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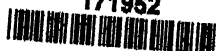
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## ERRATUM

Page 203, line 11 from foot of page: *for* Rowan, *read* Light.





# SEVEN PAPERS IN GENETICS AND PHYSIOLOGICAL GENETICS OF DROSOPHILA MELANOGASTER

BY

RICHARD BLANC, WERNER BRAUN, ELDON J. GARDNER,  
RICHARD GOLDSCHMIDT, CLAUDE A. VILLEE, JR.

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OBSERVATIONS ON THE PRODUCTION OF WING  
SCALLOPING IN DROSOPHILA MELANOGASTER

BY  
RICHARD BLANC



# OBSERVATIONS ON THE PRODUCTION OF WING SCALLOPING IN *DROSOPHILA MELANOGASTER*

BY  
RICHARD BLANC

A CLEAR EXAMPLE of the application of the concept of gene action on the basis of relative rates, times, and thresholds of reaction has been advanced by Goldschmidt (1935, 1937) in his explanation of the action of vestigial and its alleles in producing scalloping of the wings in *Drosophila melanogaster*. The different vestigial alleles and their compounds may be arranged in an orderly series based on the degree of effect, as was shown by Mohr (1932), Goldschmidt (1935b), and others, ranging from the normal wings of  $vg^{n1}$  and slightly notched wings of  $vg^{n2}$  flies through the stumplike vestigial form to the mere remnants remaining in more extreme but still viable types. The occurrence of these different grades of scalloping has been described by Goldschmidt as the result of a lytic process initiated at a different period in development in each grade. The earlier the inception of destruction in ontogeny, the more does the resulting type deviate from normal.

Thus, Chen (1929), Goldschmidt (1935), and Auerbach (1936) have shown that the wing buds of  $vg$  flies are smaller than normal in the third larval instar. Furthermore, Goldschmidt (1935, 1937) found that notching of the wings of  $vg^{n2}$  flies may be first recognized 6-8 hours after the beginning of the pupal instar, whereas scalloping in more extreme types is already present at the beginning of this period.

Recently, Waddington (1940) has raised a number of objections to this explanation. He maintains that, on the contrary, the pattern of scalloping in all grades is already determined in the wing bud at the time of puparium formation and the eversion of the wing sac, and that lower as well as higher grades of notching are simply obscured at the beginning of pupation because of the extreme inflation of the wing sac at this time. This explanation implies among other things that at the moment of eversion of the wing sac and prior to its inflation, notching should be apparent in the  $vg^{n2}$  wing. It was therefore suggested by Professor Richard Goldschmidt that I reanalyze the situation in  $vg^{n2}$  and a number of other mutant stocks with particular reference to that period. The results of this investigation and its bearing on some of the points raised by Waddington, are presented here. I should like to acknowledge the assistance rendered by the personnel of Work Projects Administration, Official Project 165-1-08-73, Unit C 1.

## MATERIALS AND METHODS

Larvae of the constitutions, crossveinless cut-six ( $cv\ ct^6$ ), vestigial-notched ( $vg^{n2}$ ), vestigial-notched/vestigial-nipped ( $vg^{n2}/vg^{np}$ ), and vestigial-notched/vestigial ( $vg^{n2}/vg$ ) were raised on cornmeal-molasses-ager-yeast media at 25° C. Optimal food conditions were maintained by allowing 10-12 females to lay eggs in each bottle for not more than 2-3 hours and by periodically replenishing the supply of live yeast. Flies reaching the prepupal instar, as

measured by cessation of motion and eversion of the anterior spiracles, were removed hourly and placed on moist filter paper in Petri dishes. Then, at definite intervals the prepupae were clipped at both ends, to allow rapid penetration of reagents beneath the pupal case, and were immersed for 4-6 hours in Bouin-Allen fixative. The wings were subsequently removed, variously stained, and mounted. The stain mostly used was Delafield's haematoxylin. Various modifications of the silver impregnation method were also employed in order to stain contours of cells and sinuses.

The description of development in the normal wing is derived from a study of material previously gathered under comparable conditions. The analysis of development in mutant types in the pupal period, is, for the most part, based on a study of slides prepared by Professor Goldschmidt and used by him in his papers on the subject.

#### THE PREPUPAL PERIOD

Eversion of the wing sac occurred at about the beginning of the sixth hour of prepupal life, as was previously observed by Chen (1929), Auerbach (1936), and Robertson (1936). Although this time may vary slightly, no external signs of wings were seen in prepupae fixed earlier than the 5-6 hour period. The everted wing at this stage is a thick organ, more or less elliptical in cross section, with the proximal portion displaying a few shallow folds. Traces of the prepupal wing venation may already be seen; central lacunae are clearly demonstrated in some specimens. A rapid expansion of the wing soon begins, resulting in a flattened, broader, longer form. The upper and lower wing surfaces, each a single layer of epithelial cells, are closely approximated, and the central and marginal lacunae become distinct. The columnar epithelium becomes cuboidal as expansion progresses. At 9-10 hours of prepupal life, the distal part of the wing has expanded to about its full extent, the epithelial layers become separated from each other, and the lacunae are largely obscured. The proximal part of the wing still shows signs of folding, but by the time pupation occurs, at about 12 hours, the whole wing has become an inflated but flattened sac with a narrow, fairly even border. At this stage, lacunae are no longer visible. In general, this description agrees with Waddington's. However, he uses the term "eversion" to include the period starting when the wing sac first begins to form by inpocketing of the wing ridge, and ending when the wing appears externally, having broken through the "peripodial" membrane. Nevertheless, the usual procedure, which is followed in this paper, is to consider that eversion is limited to the time of external appearance.

Conclusions drawn from comparison of subsequent wing shapes with that of the fully inflated bag stage may sometimes prove misleading. As Waddington has suggested, re-entrant angles tend to be obscured by the inflation. Thus,  $vg^{ao}/vg^{ap}$  wings are often distinctly notched at 6 hours of prepupal life, but seem for the most part normal at 12 hours. Similarly, as Waddington has already pointed out, the marked distal notch of the Xa prepupal wing becomes less pronounced as inflation proceeds. No structures are then apparent by means of which different regions of the wing might be recognized and studied for deviations from normal; the sharply defined lacunae of the younger wing

are no longer visible. At 6 hours, however, the newly everted wing is little expanded and usually has the prepupal venation clearly demonstrated. Thus, study of these wings should afford critical evidence of the extent to which scalloping is already present in different mutant types prior to pupation.

Examination of newly everted wings of  $vg^{no}$  and  $ct^6$  flies affords no evidence of scalloping. In plates 1 and 2 typical examples are presented, varying in size from the minute  $ct^6$  wing of figure 1 to the already much expanded  $vg^{no}$  wing of figure 6. *The wings are all of normal shape, with a margin giving no indication of degeneration.* Particularly striking are the  $vg^{no}$  wing of figure 5 and the silver impregnated  $ct^6$  wing of figure 3. In each of these wings, a well-defined submarginal sinus sets off a distinct and apparently complete border at the distal end. Silver impregnation has also accentuated the normal appearance of the  $vg^{no}$  wing in figure 6, in which the evenly distributed cells are all clearly outlined.

Waddington has stated that the  $ct^6$  wing is narrower than normal. No such conclusion could be drawn from the present material, neither for the bag stage nor for the younger uninflated stage. However, the wide range in size exhibited by young prepupal wings makes exact comparison difficult. Waddington's photomicrograph of a narrow  $ct^6$  wing presents an atypical appearance, such as is occasionally found in other wings as well, owing, perhaps, to distortion from treatment.

Examination of newly everted wings of  $vg^{no}$  and  $ct^6$  flies therefore favors Goldschmidt's hypothesis of normality at this stage and militates against Waddington's assumption that scalloping is already present. However, study of the same stage in more extremely scalloped types does not so easily favor a definite conclusion. According to both hypotheses, extreme types should already show scalloping at eversion. Prepupal wings of  $vg^{no}/vg$  and  $vg^{no}/vg^{np}$  flies are illustrated (see pl. 3, figures 7-10 and pl. 4, figure 11).

The abnormality of the  $vg^{no}/vg$  wings is evident and is not obscured by inflation. The tiny wing of figure 9 shows a pronounced lobing. Figure 10 illustrates a wing which is not only scalloped along the lateral margins but is truncated at the point where the central lacuna divides into two. The much inflated wing of figure 11 is still extremely scalloped.

Wings of  $vg^{no}/vg^{np}$  flies are less scalloped. In figure 7, a lateral margin (the posterior?) is somewhat scalloped. The wing in figure 8 shows a more pronounced scalloping of the lateral margin as well as distal scalloping. In this wing, the longitudinal sinuses seem to run to the edge in the notched region, with no distal submarginal sinus present. The marked variation observed in  $vg^{no}/vg^{np}$  wings at this stage may be due to sexual dimorphism, the occurrence of which is demonstrated in the wings of the imagines. At the fully expanded stage of early pupation an appearance of normality is generally obtained for the distal margin, but no definite conclusion can be reached for the lateral margins.

Comparison of the prepupal wings with the extremely scalloped pupal wings of  $vg^{no}/vg$  and  $vg^{no}/vg^{np}$  flies suggests that the scalloping process is not complete at eversion in these forms, but continues into the pupal period. Never-



theless, this statement cannot be made with certainty, since the fate of any particular wing region cannot be followed. What is needed is a recognition of structures common to prepupal and pupal wings. If, for example, prepupal and pupal venation could be homologized, different regions of the wing could be followed through a series of stages. An attempt is now being made to demonstrate by appropriate staining techniques any prepupal structures which persist through the inflated bag stage into the pupal period. An alternative or supplementary method for determining possible wing loss is to measure wing area, count the number of cells per unit area, and calculate the increase or decrease in total number of cells.

### THE PUPAL PERIOD

Study of the pupal period indicates that at least part of the scalloping process occurs during that stage in development. The following presentation is, in part, an amplification of Goldschmidt's previous (1935, 1937) analysis.

For purposes of comparison with the development of mutant wings, a description of normal development is given. Pupation occurs after a prepupal period of about 12 hours. At this stage the wing sac has ceased expanding, has surrounded itself with a chitin sheath secreted by the wing epithelium, and may even be slightly retracted within the confines of the circumambient sheath. This retraction is the first sign of the progressive deflation of the wing sac and approximation of the epithelial layers, which are characteristic phenomena of wing development in the first day and a half of pupal life. At 18 hours, the deflation is already pronounced. Not only is there considerable retraction with reference to the chitin sac, but the marginal hypodermis has formed a rather thick deep-staining border, owing, in part at least, to approximation of the upper and lower layers of the epithelium. The marginal epithelial cells, which are distinctly cuboidal at 12-15 hours, have now begun to assume a columnar appearance. Further evidence of contraction or condensation are the dark streaks which appear in the distal area of the wing in regions which correspond to the subsequent position of longitudinal veins 2 and 3. At 21 hours, the distal ends of veins 2 and 3 are clearly represented by lacunae which connect with the distal submarginal sinus. The similarity in appearance between this stage and the slightly inflated prepupal wing with respect to pattern and position of these sinuses is highly suggestive of possible identities. Within the next few hours the submarginal sinus extends around the periphery of the wing, and the complete vein pattern of the adult wing becomes visible. At 36 hours, the wing has finished retracting and presents a pattern similar to that of the adult wing. Subsequent to this stage, the wing enlarges and undergoes a characteristic folding process. This sequence of events in the normal wing has been briefly described and illustrated elsewhere (cf. text and plates in Goldschmidt, 1937).

During the 12-18 hour period, the  $vg^{ao}$  wing develops normally. Evidence of the distal notch may be first found when the thick border of the 18-hour stage appears. At this time the distal margin may be (1) normal; that is, not notched, with the marginal layer of uniform thickness; (2) slightly notched, but with no

noticeable thinning of the marginal layer; (3) slightly notched, with a noticeable thinning, or (4) apparently interrupted; that is, with unstained regions. The last mentioned type, with an incompletely stained or interrupted border, is particularly suggestive of a loss of material. Figures 17 and 18 present two such wings, with incomplete staining in the region of the incipient notch. Figure 18, shows two notches, each with an unstained region.

The sequence of events with respect to the distal submarginal sinus is also indicative of loss. Young pupal wings show an uninterrupted submarginal sinus. As development proceeds, some notched wings still possess a complete sinus (fig. 14), whereas others display a pronounced notch, with the submarginal sinus apparently interrupted in that region (fig. 15). Older wings show a stronger notch with the sinus obviously incomplete (fig. 16). The natural inference is that a structure that has been formed is subsequently destroyed. There is no evidence that the canal has collapsed because of retraction in that area. Certainly the burden of proof rests on those who deny that notching is due to actual destruction of tissue at this stage.

A similar sequence may be observed in *ct*<sup>6</sup> wings, as is demonstrated in plate 7. Figure 20 shows a young pupal wing with lacunae just becoming apparent; the submarginal sinus is continuous and the marginal epithelium forms an ample border. In figure 21, the epithelial border is appreciably reduced, but the submarginal lacuna is still complete. In figure 22, however, submarginal lacuna and epithelial border are entirely missing in the distal part of the wing.

In occasional favorable specimens, a sheathlike structure is found closely circumscribing the posterior end of the deflated *vg*<sup>ao</sup> wing (pl. 6, fig. 19). The identity of this structure is uncertain. It may be a secondary chitin sheath or a chitinous remnant of dissolved or retracted cells, with the area of the notch filled with a coagulum of some sort. It may be a nonchitinous secretion. But the important point is that, whenever seen, this structure preserves the outline of a normal wing over the notched area.

Pupal wings of markedly scalloped types show abnormalities even in the inflated stage. Irregularity in shape, possibly involving different resistances to blood pressure during inflation, indicates that some scalloping is already present. But characteristic and suggestive is the presence of dense-staining marginal areas where the most pronounced scalloping or loss is to become apparent. The subsequent disappearance of these epithelial masses would seem to be most easily explained by a hypothesis of actual cell loss by lysis, a process frequently found in insect metamorphosis. Wings of this type have been pictured and described at length by Goldschmidt (1935, 1937).

The most striking evidence for degeneration is furnished by the pupal development of the vestigial wing. In the inflated bag stage the wing is already highly abnormal in appearance. It is reduced in size and divided into two lobes—a proximal lobe that includes the main body of the wing sac, and a terminal lobe that is attached, sometimes rather tenuously, to the rest of the wing. Whether this terminal lobe is always represented in the retracted wing is uncertain. The retracted wing often has a terminal knob which closely resembles in general form the terminal lobe. This parallelism has been stressed

by Waddington as evidence that the terminal lobe is never pinched off or lost but is retracted with the rest of the wing. However, this similarity between knob and lobe is often rather vague at best. Even figure 23, though fairly typical, does not clearly signify that only retraction is involved.

The terminal portion of the retracting wing varies: it may be a flat triangular mass of epithelium, with regions which do not stain and may actually be in process of dissolution (figs. 26, 27); it may be a solid knob of epithelial cells; occasionally (fig. 24) a portion of this terminal mass is almost detached. Again, the knob may not be solid but may possess a blood sinus which is often seemingly unconnected with the blood system of the rest of the wing. In such wings, the basal portion of the wing retracts normally, as deflation proceeds, but the terminal part often remains swollen, as though the fluid in the blood cavity were trapped, thus preventing further deflation. Suggestive in this respect is the appearance of many  $vg^{no}/vg$  wings, in which there is a distinct terminal lobe. With partial deflation, central blood sinuses appear in this terminal portion. The whole wing may continue to contract to the stage shown in figure 12. Or, as in figure 13, the distal sinuses may be unconnected with the blood lacunar system of the rest of the wing and appear bloated, as though charged with fluid.

The terminal portion of the vestigial wing is sometimes slightly lobed, and organically related to the main body of the wing. However, this form is abnormal in that the lobe, unlike the rest of the wing, remains inflated and forms no lacunar system (figs. 25, 28, 29). Figure 25 may represent an early stage in this process. The wings in figures 28 and 29 are particularly interesting. The basal part, or wing proper, is normal; in fact, the proximal part of the 44-hour wing (fig. 29) has begun to fold in the usual fashion. In contrast, however, the distal portion is strongly suggestive of the 18-hour stage, even to the central longitudinal strands which are the precursors of longitudinal veins 2 and 3 in a normal wing. The many phagocytelike cells in these undifferentiated ends suggest phagocytosis as a factor in the development of wing form. The younger, 34-hour wing (fig. 28) shows numerous large free cells invading this region. In the 44-hour wing, these cells are fewer, with the nucleus polarized at one end and the rest of the cell filled with a yellowish material. Nevertheless, phagocytic action does not seem to be the primary mechanism of histolysis in the vestigial wing, since it can be demonstrated only in these cases of developmental arrest. Otherwise ordinary lysis by enzymatic digestion and liquefaction seems to be the rule.

## DISCUSSION

On the basis of the present evidence, Waddington's objections to Goldschmidt's explanation of different degrees of scalloping would not seem to hold. Waddington has pointed out that the inflation of the bag stage tends to obscure scalloping and may make lesser grades appear normal at this time. This fact has been demonstrated for  $vg^{no}/vg^{sp}$  and  $Xa$  wings. However, it does not follow that all lesser grades are notched before inflation; for example,  $vg^{no}$  and  $ct^6$  wings. Nor does Waddington's contention hold that lower grades of distal notching may not be obvious in the tiny prepupal wing. The  $vg^{no}$  and  $ct^6$  wing

margins at this stage are demonstrably intact. This is particularly evident in wings with a well-defined distal marginal sinus. Furthermore, my observations do not bear out his contention that in the uninflated and inflated stages, the  $ct^s$  wing is narrower than normal. And even in the uninflated stage, the obviously scalloped  $vg^{no}/vg^{np}$  and  $vg^{no}/vg$  wings seem to be more nearly normal than they are subsequent to inflation.

Waddington has stressed the indirect nature of the evidence for histological degeneration in the pupal period. It is true that no indications of dying cells or pycnotic nuclei are forthcoming. Yet pycnosis is not a necessary concomitant of histolysis in insect tissue. Indeed, dead cells may appear normal until they simply dissolve away by autolysis or are attacked and digested by phagocytes. (See Wigglesworth, 1939, for references on histolysis in insects.) That an autolytic process is at work is strongly suggested by the failure of some areas to stain (cf. pl. 6, figs. 17 and 18; pl. 9, figs. 26 and 27). The presence of masses of cells along the wing margin of strongly scalloped types raises the question of their prospective fate. If, as Waddington assumes, these cells are not destroyed but are retracted into the body of the wing, how does this rearrangement occur without upsetting the normal cell distribution which is apparent later? The subsequent disappearance of differentiated regions such as margin and submarginal sinus in  $vg^{no}$  and  $ct^s$  wings, strongly suggests destruction.

Waddington pays particular attention to the vestigial wing. He states that the main evidence for its degeneration is: (1) the constricted lobe or knob sometimes appears about to break away from the end of the inflated sac to which it is attached; (2) the area within the chitin sac occupied by the wing rudiment is much smaller than the same area in the normal wing. He remarks with respect to the first point that no evidence of a cast-off knob has been observed and that, also, different degrees of retraction of the knob into the body of the wing may be found. Concerning the second point, he attributes the small size of the rudiment to two factors: (a) the wing base is more contracted than the rest of the wing (a vestigial wing represents only the basal part); (b) abnormal contraction results from a dumpy phenomenon, as indicated by distortion of the vein pattern. As already pointed out, retraction of the terminal knob into the body of the wing may be apparent rather than real. But the terminal portion of the wing may very well degenerate at different times, and the time of this degeneration may depend on the degree to which the blood lacunar system of the wing extends uninterruptedly into the distal part. This would explain the variety of types obtained, including the interesting examples shown in plate 9, figures 28 and 29, in which the wing appears as an organic whole with no part lost but with development arrested in the distal portion. Great contraction of the wing base does not explain the extent of lengthwise contraction of the vestigial wing. Furthermore, a dumpy process seems unnecessary in explaining distortion of the vein pattern; a high degree of scalloping superimposed on normal retraction may well be enough to cause abnormal tensions with resulting distortion.

Since the evidence still seems to favor a concept of progressive scalloping by degeneration, the more complicated alternative hypothesis advanced by

Waddington, apparently on the basis of analogy with amphibian embryology, is not acceptable. Indeed, considering that the important function of histolysis in insect development has been generally recognized ever since Weismann (1864), there should be no reluctance to accept it as a factor in determination of wing pattern. Formation and subsequent histolysis of wing tissue have been recorded for a number of insects, notably by Keilin (1913) for *Belgica antarctica*, by Hopkins for *Pryxia scabiei*, and by Stange for *Melophagus* (Hopkins and Stange cited in Bezzi, 1916).

Except for  $ct^6$ , Waddington's observations and mine are in close conformity. The disagreement rests on difference of interpretation of the developmental phenomena. Waddington confined himself largely to a study of extremely scalloped types, but it is the behavior of the lower grades in the prepupa which provides a critical test of his concept.

Waddington's hypothesis meets with a number of other difficulties. If scalloping depends on the position of the wing fold relative to the venation system, then scalloping pattern is completely determined, excluding a later secondary process, by the time of puparium formation. Therefore heat treatment of prepupae and pupae of normal flies should not produce scalloping. Indeed, Waddington cites Goldschmidt (1939) for a statement that the sensitive period for production of scalloping phenocopies is situated at the end of the third larval instar. Unfortunately, this statement is not correct. Fricisen's (1936) work shows that the effective period for wing scalloping by X-ray treatment occurs early in the third larval instar, but Goldschmidt's (1935a) data point to postlarval life as the time for positive results with temperature treatment.

If the scalloping pattern is already determined in the larva, then alteration of relative rates of reaction subsequent to this stage, by such an agent as temperature, will not change the pattern. Yet, evidence from preliminary experiments of my own suggests that changes of temperature in the prepupal and pupal periods lead to a definite increase in scalloping in  $vg^{no}$  and  $vg^{no}/vg$  flies. If Waddington wishes to account for such results within the scope of his hypothesis, he will have to admit the existence of a postlarval lytic process. This explanation is possible, of course, but the necessity for considering it removes one of the reasons for his original concept, to wit, the supposed lack of evidence for lysis in the prepupal and pupal periods.

Observations of the wing fold actually permit no definite conclusions regarding the occurrence of scalloping and the nature of vein determination; Waddington's description and drawings are not clear on these points. Lack of clarity of all except the grossest details necessarily limits the value of the study of the whole, unsectioned, wing bud. An accurate description of the process of folding awaits more refined methods of observation.

Even apart from such points, it is difficult to conceive of the mechanics of the process suggested by Waddington. Indeed, he himself comments that his idea "may appear somewhat fantastic and unlikely."

## SUMMARY

(1) Newly everted, uninflated  $ct^6$  and  $vg^{no}$  wings show no signs of scalloping but appear normal, with distal submarginal sinus and marginal epithelium demonstrably intact.

(2) The more extreme  $vg^{no}/vg^{np}$  and  $vg^{no}/vg$  wings already show scalloping at this time, but to a lesser degree than in the later deflated pupal stage.

(3) Lysis is indicated at 18 hours in  $vg^{no}$  wings by the occasional failure of the distal marginal epithelium to stain in the region in which the notch subsequently appears.

(4) As notching becomes more prominent in pupal wings of  $vg^{no}$  and  $ct^6$  flies, the distal submarginal sinus tends to be suppressed.

(5) In  $ct^6$  wings, the anterior margin is apparently complete in young pupal wings but is distinctly reduced in thickness after deflation.

(6) The sheathlike structure sometimes found around the posterior end of the deflated  $vg^{no}$  wing shows a normal contour in the region of the notch.

(7) In young pupal wings of more extremely scalloped types marginal masses are present whose later disappearance is most easily explained by lysis.

(8) The similarity in form between the distal knob of the retracted  $vg$  wing and the distal lobe of the chitin sheath is often vague enough to cast doubt on retraction as being the sole phenomenon involved.

(9) Pupal degeneration of the distal region of the  $vg$  wing is indicated in the series of forms varying from those with a distal flat mass of partially nonstaining epithelium to those in which the phagocyte-filled distal portion has remained relatively undifferentiated while the proximal part has continued to develop into the definitive wing.

(10) Histolysis is a common phenomenon in insect development and has even been observed in the formation of the wing in several kinds of insects.

(11) The pattern of scalloping can be altered by temperature treatment in postlarval stages, as is indicated by the production of notched wings as phenocopies in normal flies and by the enhancing of scalloping in  $vg^{no}$  and  $vg^{no}/vg$  flies.

(12) No clear explanation of the mechanics of the process suggested by Waddington is apparent.

(13) On the basis of the evidence thus far presented, there seems to be no justification for rejecting Goldschmidt's explanation in favor of Waddington's more complicated hypothesis.

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## PLATES



## PLATE 1

Stages in the development of the scalloped wing of *Drosophila melanogaster* Prepupal wings, cv ct<sup>6</sup>.

Fig. 1. Age, 6 to 7 hours. Silver impregnation  $\times 156$

Fig. 2. 6 to 7 hours Ag impreg.  $\times 154$

Fig. 3. 6 to 7 hours. Ag impreg.  $\times 111$



3



2



1

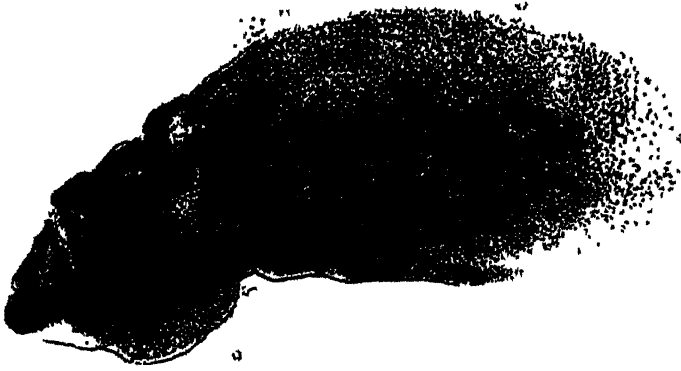
## PLATE 2

Stages in the development of the scalloped wing of *Drosophila melanogaster*. Prepupal wings,  $v_8^{no}$ .

Fig. 4. 6 to 7 hours. Delafield's haematoxylin.  $\times 140$ .

Fig. 5.  $5\frac{1}{4}$  to  $6\frac{1}{4}$  hours Iron haem  $\times 142$ .

Fig. 6. 6 to 7 hours. Ag impreg  $\times 149$ .



### PLATE 3

Stages in the development of the scalloped wing of *Drosophila melanogaster* Prepupal wings,  $vg^{no}/vg^{np}$  and  $vg^{no}/vg$

Fig 7 5 to 6 hours Del haem  $\times 141$

Fig 8 5 to 12 hours Del haem  $\times 92$ .

Fig 9 6 to 7 hours Ag imprec  $\times 133$

Fig 10 6 to 7 hours Del haem  $\times 143$



10



9



8



7

## PLATE 4

Stages in the development of the scalloped wing of *Drosophila melanogaster*.  $vg^{no}/vg$  wings.

Fig. 11. 6 to 7 hours. Del. haem.  $\times 146$ .

Fig 12. 40 hours. Iron haem.  $\times 78$ .

Fig. 13 40 hours. Iron haem.  $\times 107$ .



13



12



11



## PLATE 5

Stages in development of the scalloped wing of *Drosophila melanogaster*. Pupal wings, vg<sup>no</sup>. Del haem.  $\times 92$

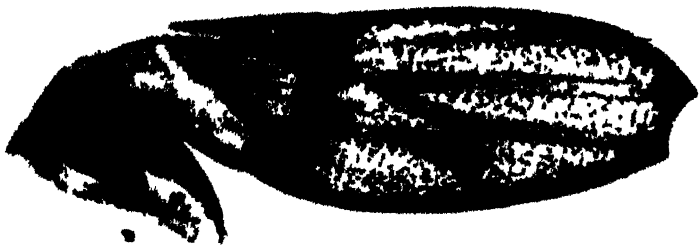
Fig 14. 30 hours

Fig 15. 28 hours.

Fig. 16. ?? hours.



16



15



14

## PLATE 6

Stages in the development of the scalloped wing of *Drosophila melanogaster*. Pupal wings *vg<sup>no</sup>*. Del. haem.  $\times 92$ .

Fig. 17. 28 hours.

Fig. 18. 28 hours.

Fig. 19. 32 hours.



19



18



17

## PLATE 7

Stages in the development of the scalloped wing of *Drosophila melanogaster*. Pupal wings cvct Delheim  $\times 92$

Fig. 20 22 hours

Fig. 21 32 hours

Fig. 22 32 hours



22



21



20

## PLATE 8

Stages in the development of the scalloped wing of *Drosophila melanogaster*. Pupal wings, vg  $\times$  139.

Fig 23. 30 hours. Osmium fixation

Fig. 24. 30 hours. Del. haem.

Fig 25. 34 hours Del. haem



25



24



23



## PLATE 9

Stages in the development of the scalloped wing of *Drosophila melanogaster* Pupal wings var. Del haem  $\times 92$

Fig. 26 24 hours

Fig. 27 26 hours.

Fig. 28 34 hours

Fig. 29 46 hours



29



28



27



26



**PHENOCOPIES AND X RADIATION IN  
DROSOPHILA MELANOGASTER**

**BY**

**RICHARD BLANC AND WERNER BRAUN**



# PHENOCOPIES AND X RADIATION IN *DROSOPHILA MELANOGASTER*

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## I. THE RELATIONSHIP BETWEEN AMOUNT OF X RADIATION AND PRODUCTION OF PHENOCOPIES

THE OCCURRENCE of nonheritable phenotypes which resemble known genetic characters has been frequently described, but their significance was first recognized by Goldschmidt (1935), who maintained that under proper conditions phenocopies—his term for these morphoses—could be found for all mutant types. Phenocopies in *Drosophila melanogaster* have been produced by a number of investigators with a variety of agents: Goldschmidt (1929, 1935), Jollos (1930, 1933), Plough and Ives (1932, 1935), Child, Blanc, and Plough (1940), and others, with high temperature; Gottschewski (1934), with low temperature; Geigy (1926) and Epsteins (1939), with treatment by ultraviolet light; Friesen (1936), with X radiation; and Rapoport (1939), with chemical agents. As Goldschmidt (1935) has observed, the number and extent of these phenocopies are dependent on four factors: (1) the stock used, (2) the developmental age of the flies when treated, (3) the duration or extensity, and (4) the intensity of treatment. Due attention has usually been paid in experimentation and analysis of data to the first two of these factors—the effects produced in specific stocks by treatment at definite stages in development. Changes resulting from different amounts of treatment have not been carefully measured.

Indeed, analysis of the effects of treatments differing in duration and intensity presents certain difficulties, which, when temperature is used as the agent, are well nigh insuperable. Effective dosages of temperature require an extended time of treatment. The difference between effectively different dosages is in terms of hours and thus involves different stages in ontogeny. The effects, therefore, are not strictly comparable in terms simply of amount of treatment. A similar difficulty is encountered in the use of temperatures of different intensities. The rate of development as a whole has been shown by Powsner (1935) to vary in *Drosophila* for different temperatures in the normal temperature range of the organism. If the same is true for the sublethal temperatures used in the production of phenocopies, a matter still to be investigated, different developmental processes may again be affected.

These difficulties are obviated by the use of an agent which produces a variety of effects with only slight differences in time. Such an agent is X radiation, in which variations of a fraction of a minute in treatment produce measurable differences in effect on phenotypes of *Drosophila*. This paper reports in part on the relationship between phenocopies and X radiation.

This work was done in 1938–39 while Richard Blanc was a Cramer Fellow of Dartmouth College in residence at the University of California. We wish to

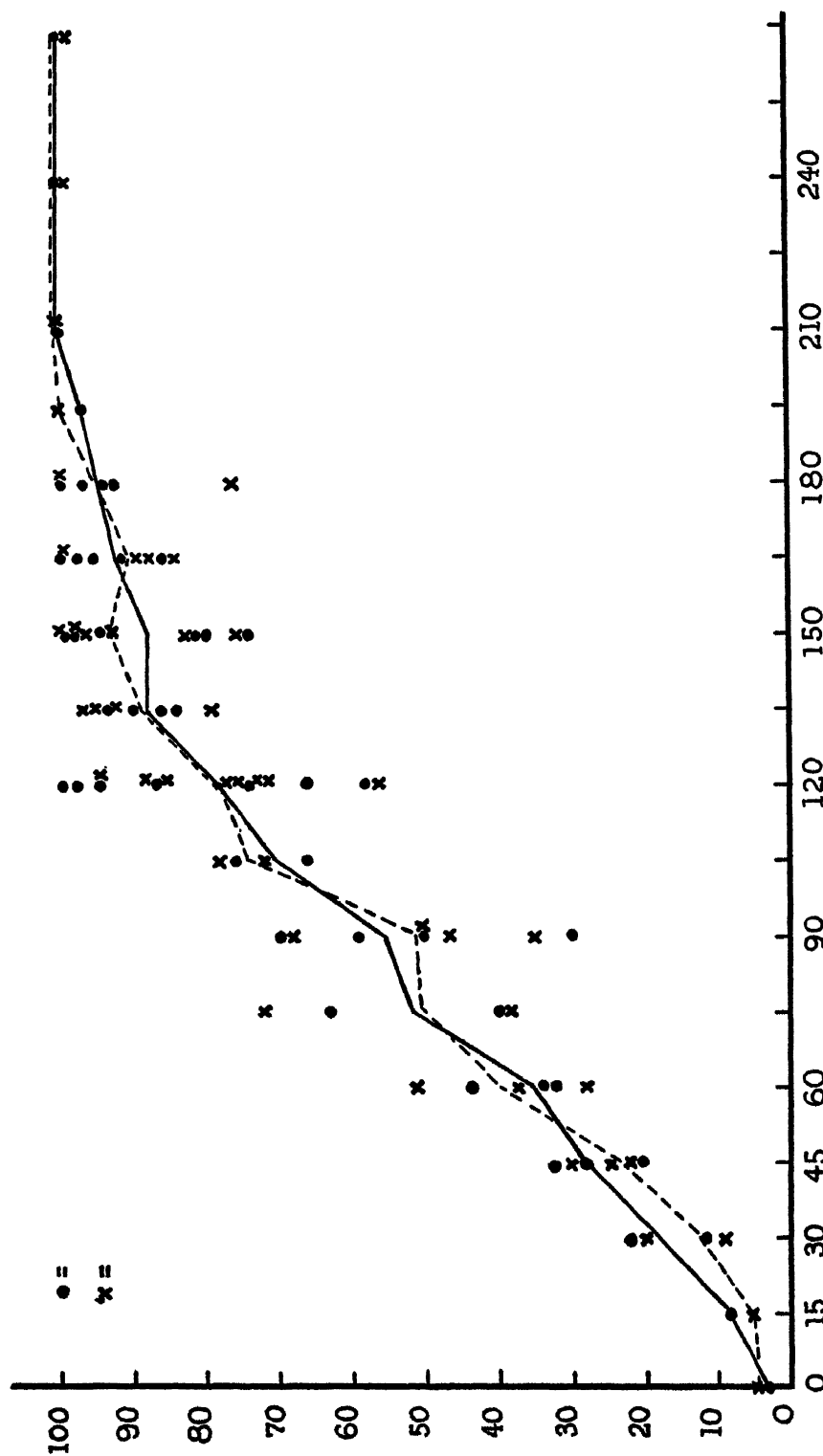


Fig. 1. Relationship between X-ray dosage and number of flies showing phenocopies. Abscissa: seconds of exposure at 940 r. units/min. Ordinate: per cent of flies showing phenocopies. Cross indicates individual experiments,  $\sigma^7$ ; dot indicates individual experiments,  $\sigma^7$ .

thank Professor Ernest Babcock, through whose courtesy we were able to use the X-ray apparatus of the Division of Genetics of the University of California; Dr. E. R. Dempster, who made several helpful suggestions; and the personnel of Work Projects Administration, Official Projects 465-03-3-192 and 165-1-08-73 Unit C-1, which rendered valuable assistance.

TABLE 1  
RELATION OF PHENOCOPY PRODUCTION TO EXPOSURE TIME

X-ray dosage in seconds	Number of pupae	Per cent of flies hatched	Per cent of flies showing phenocopies		Average number of phenocopies per imago	
			♀	♂	♀	♂
Control	278	100	3	4	.03	.04
15	176	99.4	8	5	.09	.06
30	311	98.7	17	12	.21	.17
45	278	99.6	28	23	.33	.26
60	352	96.6	35	39	.48	.48
75	234	99.1	51	51	.69	.65
90	233	94.0	55	51	.79	.73
105	186	90.3	70	74	1.13	1.10
120	500	94.0	78	78	1.56	1.52
135	427	88.8	88	88	1.97	2.11
150	676	84.2	88	93	2.21	2.25
165	595	63.0	92	91	2.60	2.69
180	599	49.6	95	95	3.07	3.21
195	249	20.9	97	100	3.47	3.77
210	117	13.7	100	100	(4.00)	(3.44)
240	65	4.6	100	100	....	....
270	97	2.1	100	100	....	....

## MATERIAL AND METHODS

The flies used were from an Oregon-R stock which had been inbred for three years. They were raised in large refrigerator dishes, and all young pupae which showed large longitudinal tracheae were transferred daily to Petri dishes and exposed to X-ray treatment. As the longitudinal tracheae usually disappear at about 12 hours after puparium formation (at 25° C.), and as all pupae were treated before this time, the pupal age at the time of treatment was 0-12 hours. Most of our material was treated at this stage, which has been described by Friesen (1936) as the period of greatest production of phenocopies. A Coolidge X-ray tube and aluminum filter, 1 mm. thick, were used. With the current at 100 kv. and 10 ma., and the distance between tube and pupae fixed at 9½ cm., approximately 940 r. units per minute were delivered by the apparatus. The pupae were left in the Petri dishes, and the flies which hatched were checked for a series of phenotypic changes.

Characters most frequently noted were the following: abnormal abdomen; various bristle defects, particularly absence of bristles and microchaetes; curled (up) and curved (down) wings; opaque texture of wings; a minute extra



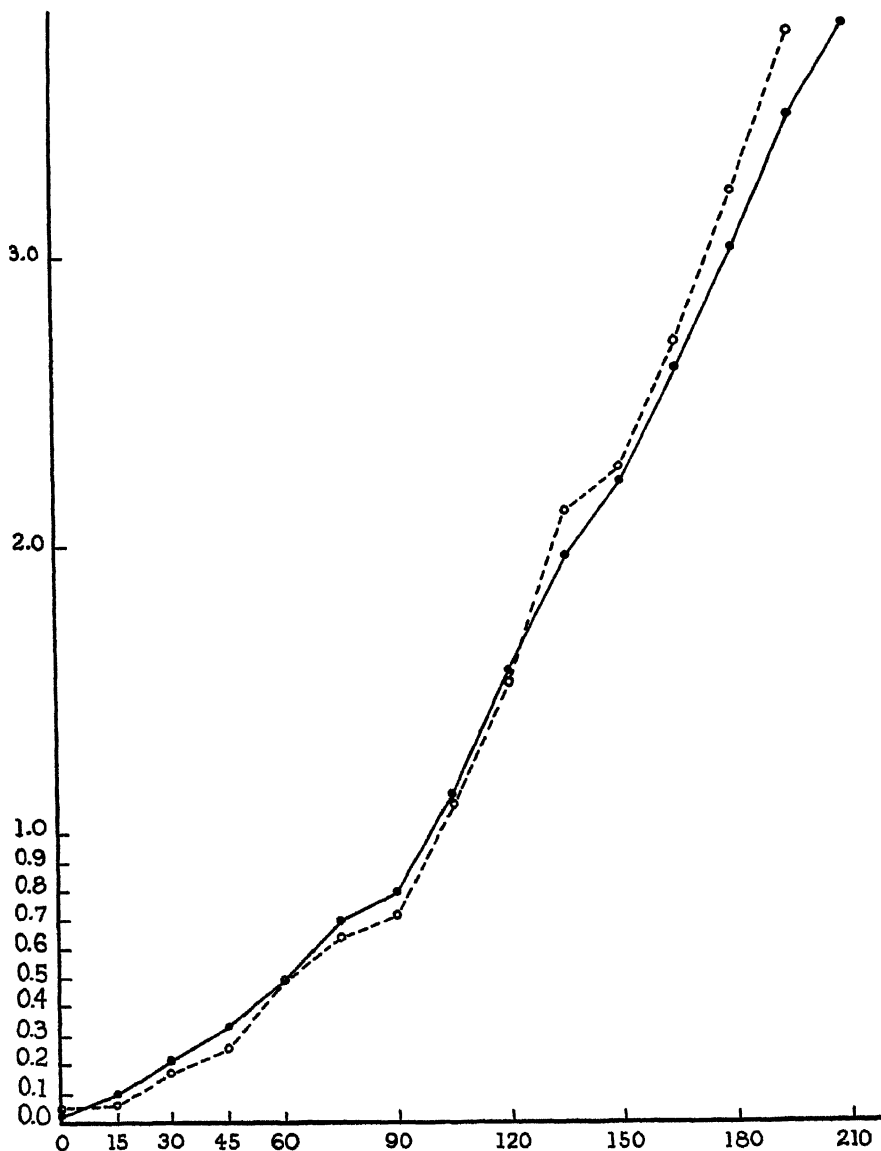


Fig. 2. Relationship between X-ray dosage and average number of phenocopies per fly. Abscissa: seconds of X radiation at 940 r. units/min. Ordinate: average number of phenocopies. — = ♀. --- = ♂.

vein leading from the distal crossvein of the wing; trident on thorax. Other phenotypes were occasionally found; they included vortex, and a number of wing effects such as crossveinless, plexus, blistered, wavy, crumpled, dumpy, and narrow. A number of these typical phenocopies are best described by the names of the mutants whose effects they closely imitate. The data on the extra vein have been disregarded, as the character already existed to a varying de-

gree in the stock. For convenience, study of bristle effects was confined to the ocellars, dorsocentrals, and scutellars. Other bristles were also affected.

A total of 5373 pupae were irradiated, exposure varying from 15–270 seconds, for the most part in 15-second intervals. This represents an increasing amount of radiation from 232–4230 r. units. The number of experiments for each interval varied from 1–8, and the resulting data were summarized for each interval (table 1).

#### THE RELATIONSHIP BETWEEN X-RAY DOSAGE AND NUMBER OF PHENOCOPIES

The relationship between X-ray dosage and number of flies showing phenocopies is represented in figure 1. The distribution of values for males and fe-



Fig. 3. Relationship between X-ray dosage and viability. Abscissa: seconds of X radiation at 940 r. units/min. Ordinate: per cent of flies hatched.

males for all experiments is plotted, and curves of the averages are drawn. The average percentages are also given in the table. Not all control flies were normal, some showing phenocopies confined to defects of the dorsocentral and posterior scutellar bristles. A 15-second exposure produced a slight but definite effect: fewer normal flies, some flies showing more than one phenocopy. A significant change occurs following an exposure of 30 seconds: a greater number of characters are affected, including wings and head bristles. With longer exposure, the percentage of flies showing phenocopies increases (see fig. 1). This relationship, shown in figure 1, is in the nature of an s curve, with an almost linear proportionality in the 15–135-second interval. At exposures of 3290 or more r. units, all flies show phenocopies.

Table 1 shows the increase in average number of phenocopies per fly with increase in X radiation. As an anterior-posterior relationship between the scutellar bristles was suggested by their essentially similar response to treatment, effects on anterior and posterior scutellars are not separately classified here. On this basis the most phenocopies in any fly was 12. Figure 2 shows the rapid

increase in phenocopies per imago as exposure increases. This increase shows a linear proportionality from 1410 up to 3290 r. units—the highest dosage for which significant data are available.

#### THE EFFECT OF INCREASED RADIATION ON VIABILITY

The decrease in viability as radiation increases is shown in figure 3. The data were obtained by calculating the ratio of imagines to the number of irradiated pupae. The curve is similar to the usual radiation mortality curves, as demonstrated for *Drosophila* eggs, for example, by Packard (1936). Our curve indicates but little mortality up to 150 seconds of exposure (2350 r. units); almost all irradiated pupae hatched. With longer exposure there is a sudden sharp drop in the percentage of flies which hatch. Starting with 210 seconds of exposure (3290 r. units), the decrease in the percentage of hatched flies is less rapid. At 270 seconds (4230 r. units) only 2 per cent of all irradiated pupae hatch.

A few of the unhatched pupae were completely undeveloped, the cases filled with an apparently unorganized, white, viscous material. Most of the flies that did not hatch appeared to develop normally, emerged partly after a day's delay, but were too feeble to emerge completely or to survive when removed.

#### DISCUSSION

The production of phenocopies by X rays probably is complex, and may occur in a variety of ways. The developing cells of a structure may be killed. A chemical or other developmental reaction may be affected directly and its function in development altered. Or, the radiation may affect a certain part of the pupa which determines or influences the development of another structure, and thus indirectly produce a change. Thus the production of phenocopies by external means may involve direct and indirect actions. Therefore, simple biological reactions need not be expected.

Although the number of flies showing phenocopies increases with greater radiation, increases are not proportional throughout, but result in an s-shaped curve (fig. 1). This contrasts with the straight lines fitted to the data on production of mutations by X radiation, as demonstrated by Timoféeff-Ressovsky (1935) and others. Interestingly, Bauer (1939) has attributed an s-curve relationship to the data on alterations in chromosomes induced by X rays.

We cannot yet explain the physical action of X radiation in producing phenocopies. Any explanation must account for the increase in rate of production of phenocopies with increase in treatment. The curve in figure 1 does not fall off as soon as would be expected of a saturation curve. Furthermore, figure 2 suggests that for up to 1410 r. units there is an increase in rate in the number of phenocopies per fly. Possibly a threshold phenomenon requiring a minimum energy level is involved.

#### II. FREQUENCIES OF INDIVIDUAL PHENOCOPIES

The phenocopies most frequently noted and analyzed in detail were: abnormal abdomen, curled (up) and curved (down) wings, opaque texture of wing, trident on thorax, absence of microchaetes; and various bristle defects, par-

ticularly absence of bristles, recorded specifically for the ocellar, dorsocentral, and scutellar bristles. The frequencies of the various phenocopies at each intensity of radiation are presented in table 2. Although most of the numerical data are not separately tabulated for males and females, the accompanying graphs are based on percentages separately computed for the sexes. Frequencies of bristle effects are tabulated on the bases of half flies; the number of flies should be doubled to compute percentages.

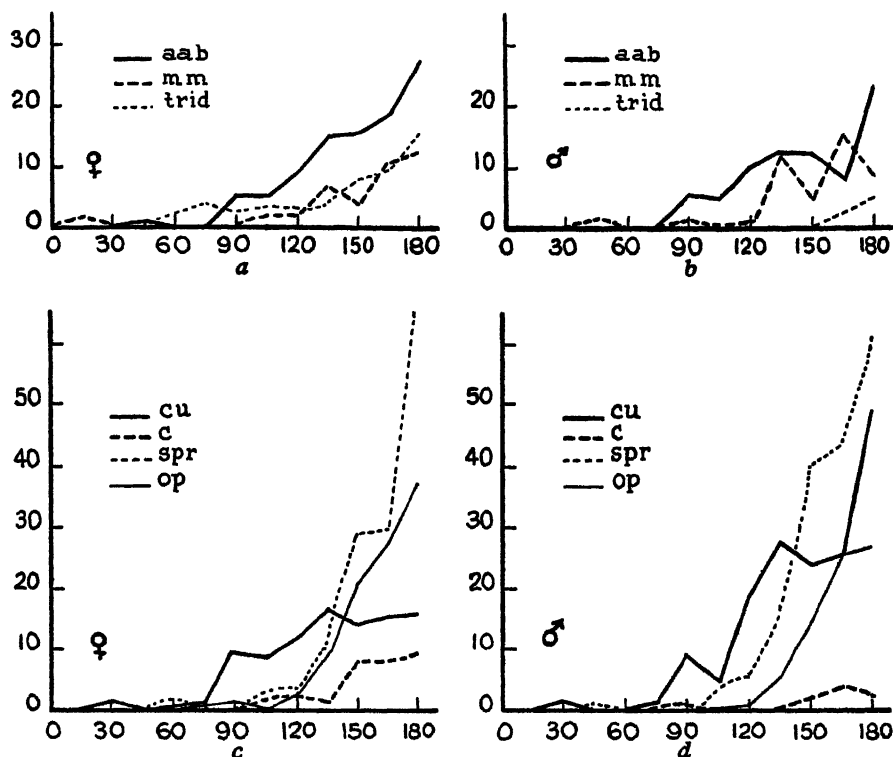


Fig. 4. Percentages of phenocopies at different X-ray dosages (abscissa: seconds of X radiation at 940 r. units/min.; ordinate: percentage of phenocopies): (a) abnormal abdomen, missing microchaetes, and trident, ♀♀; (b) same, ♂♂; (c) curled, curved, spread, and opaque, ♀♀; (d) same, ♂♂.

#### ABNORMAL ABDOMEN, MISSING MICROCHAETES, AND TRIDENT

The graphs in figure 4a represent the percentage of females showing the phenocopies abnormal abdomen, missing microchaetes, and trident at twelve different dosages, ranging from 0-2820 r. units. All three phenocopies increase similarly with increase in exposure time. The percentage of flies showing abnormal abdomen remains consistently higher than that for either of the other two phenocopies. The threshold for production of the three phenocopies is also different. Flies showing missing microchaetes first appear in significant numbers after irradiation of 940 r. units; abnormal abdomen, at 1410 r. units; and trident, at 1647 r. units.

**TABLE 2**  
**RELATION OF PHENOCOPIY TYPES TO EXPOSURE TIME**  
 (Data for bristle effects based on half flies)

X-ray dosage in seconds	Number hatched	Normal	Phenocopies ♂ and ♀													Others
			ocs	ades	pdes	ascs	pscs	cu	c	op	spr	tri	mm	aab		
0	278	270	0	6	1	0	2	0	0	0	0	0	0	0		
15	171	160	0	7	2	0	3	0	0	0	0	0	1	0		
30	305	258	9	29	6	4	3	2	1	1	1	0	0	0	1 ♀ spatula wing, 1 ♀ thorax abnormal, 1 ♂ partial cv	
45	277	207	16	36	5	21	12	0	0	1	0	0	2	1		
60	340	217	34	59	23	43	19	1	0	2	2	4	0	0	1 ♀ half thorax, 1 ♀ different abnormal abdomen	
75	232	113	36	67	19	30	20	3	0	1	0	4	0	0	2 ♀ notched, 1 ♀ cv and small round wing, spoonlike	
90	216	103	25	70	19	29	11	19	1	2	0	4	1	10	1 ♂ small and blistered wing, 1 ♂ 1 short wing	
105	168	47	42	72	18	39	22	11	1	0	5	2	1	8	1 ♀ extra vein in III, 1 ♂ dp	
120	470	104	162	184	79	165	118	73	4	7	21	6	4	44	1 ♀ narrow wing, 1 ♀ spatula wing, 1 ♂ crumpled wing, 1 ♂ soft wing, 1 ♂ short wing, 2 ♂ uplifted wings, 1 ♂ right eye glazed, 1 ♂ plexus	
135	379	43	146	265	49	141	69	81	3	8	52	13	31	56	1 ♀ truncate, 1 ♂ blistered, 1 ♂ plexus, 1 ♂ vortex	
150	567	57	193	366	67	234	83	102	26	100	193	24	21	78	1 ♀ crumpled wing, 1 ♀ soft wing, 1 ♂ bent wing, 1 ♂ dumpy	
165	380	34	142	288	50	151	65	83	24	71	130	23	47	56	2 ♀ lifted wings, 8 ♀ crumpled wings, 1 ♂ microchaetes extremely disarranged, 2 ♂ crumpled wings	
180	260	15	104	192	43	125	63	80	16	62	165	27	29	65	2 ♀ crumpled wings, 1 ♀ growth from left wing, 1 ♀ slightly vortex, 1 ♂ slightly vortex, 2 ♂ blistered, 1 ♂ crumpled wing	

Figure 4*b* represents the production of the same phenocopies in males. Comparison with figure 4*a* shows some similarity for abnormal abdomen, less for missing microchaetes (threshold at 1880 r. units), and a striking difference for trident. Trident phenocopies in males are seldom produced, and then only after a treatment of at least 2820 r. units. Differences in response of males and females have been noted and discussed by other investigators; the present results are of no unusual significance.

#### PHENOCOPIES OF THE WING

In figure 4*c* are graphs for four different phenocopies affecting the structure and position of the wing of females. Curled flies first appear in significant numbers at 1410 r. units and then increase in number like the three phenocopies in figure 4*a*. The phenocopy curved is produced less frequently, with a material increase at 2350 r. units. A significant number of flies with spread and opaque wings appear at 1650 r. units, and their number increases rapidly with increasing radiation.

Figure 4*d* shows the corresponding graphs for the males. Chief differences are the much lower rate of increase of curled wings and slightly greater increase of curved wings.

#### BRISTLE EFFECTS

Because of their possible bearing on bristle pattern, effects on macrochaetes received special attention. Complete records were kept of the effects of treatment on each of the ocellars, dorsocentrals, and scutellars. Effects on other head and thoracic bristles were noted but not recorded. A number of different phenotypes were distinguished; these were listed as forked, bent, fused, thin, small, hairlike, vestigial, and missing. When bristles were missing, the pores were present, reduced, or absent. With the exception of forked, bent, and fused bristles, which were few, the various effects clearly form a simple series of bristle deficiencies. This suggested that most, if not all, of the types were but modulations of an essentially similar effect and could be tentatively assumed to be due to alterations of closely related processes. On this basis, the data have been summarized without regard to variety of effect.

The bristle data are presented in table 2 as total numbers of half flies with a particular bristle affected. We made the assumption that there is no left-right correlation in a genetically homogeneous stock raised under similar environmental conditions (see Plunkett, 1926). Indeed, lack of such correlation has been taken as a measure of the genetic homogeneity of a population (Child, 1935). Because the Oregon-R stock we used has been inbred for some time and probably was fairly homogeneous, any left-right correlation would reasonably seem to measure common environmental change and not the effect of one side on the other (see also Margolis, 1935; Neal, 1940). The half fly has therefore been taken as the unit.

Figure 5*a* gives the percentages of ocellar bristles affected by each dosage, plotted separately for left and right sides, and shows clearly the similarity of response of the two sides. Since this is typical of the other bristles also, the data for left and right anterior and posterior dorsocentrals and anterior and

posterior scutellars have not been plotted separately. Thus the graphs of figures 5b and 5c are based on percentages per half fly.

The data for males and females are separately plotted, and the similarity of response in the two sexes is clearly demonstrated. Bristle effects occur early; the dorsocentrals and posterior scutellars are affected even among the controls, and at  $\frac{1}{2}$  minute of radiation all the different bristles some effect.

Although the bristles respond alike in early occurrence of effects, similarity of effect in males and females, and parallelism of response of left and right

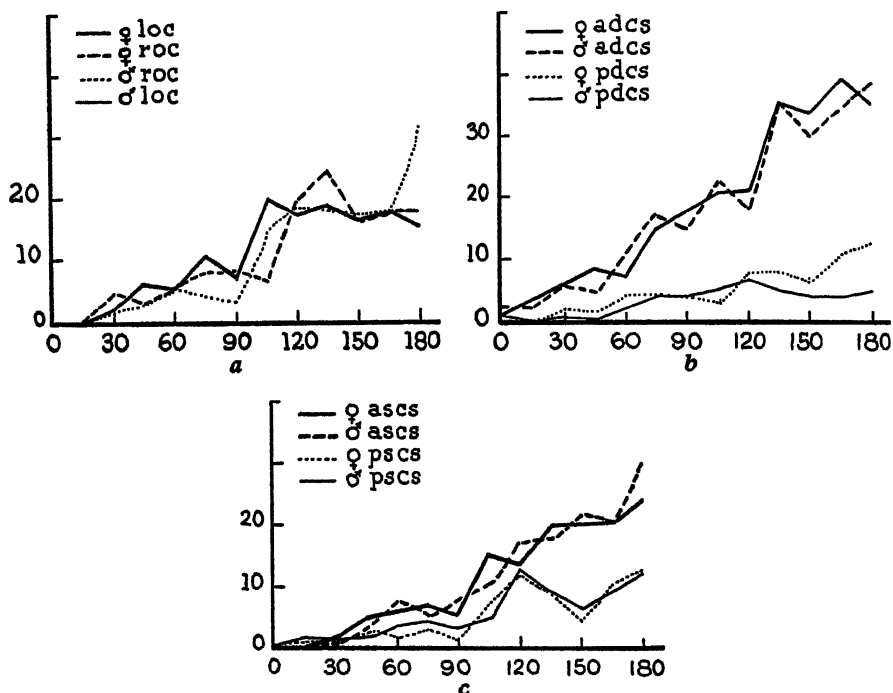


Fig. 5. Percentage of bristles affected by different X-ray dosages (abscissa: seconds of X radiation at 940 r. units/min.; ordinate: mean percentage affected): (a) ocellar; (b) anterior and posterior dorsocentrals; (c) anterior and posterior scutellars.

sides, they differ distinctly *inter se* in rate of increase of effect. This is most striking in the dorsocentrals, in which there is a great increase in effects anteriorly, but only a slight increase posteriorly (see fig. 5b), suggesting that the anterior and posterior dorsocentrals are not closely associated in development, at least so far as the X-ray affected stages are concerned. In contrast, as demonstrated in figure 5c, the anterior and posterior scutellars show strikingly similar reactions, differing only in degree, suggesting that the same or closely associated developmental processes are affected. Thus the dorsocentrals may be said to belong to separate, and the scutellars to the same or closely related, reaction systems.

We attempted to analyze statistically the anterior-posterior and left-right relationships between pairs of bristles with respect to response to varying

degrees of treatment. Unfortunately, the results were inconclusive owing to inadequacy of the data, and show only that the relationship between any two bristles and their response may be expressed as a straight-line relationship. The statistical method and results are in the appendix.

## DISCUSSION

Friesen (1936) reported roentgenmorphoses produced in *Drosophila* by exposure to X radiation of 2000 and 4000 r. units. Since his experimental method differed from ours, exact correspondence between the results cannot be expected. However, comparison shows a similarity. The periods which Friesen records as "0-24 hours after pupation" and "96-120 hours after egg-laying" apparently include the prepupal stage. By treatment at this stage he also obtained spread wings, opaque (rough) wings, abnormal abdomen, and reduction of bristles. In addition, he records a large percentage of rough eyes, a phenocopy we did not look for. He did not obtain curled wings. The conflict in results may be due to different experimental conditions or, more probably, different stocks. For Friesen obtained different responses from his two wild stocks of *Drosophila*, and Lobashov and Solodovnikov (1939) found differences in sensitivity to X radiation in a number of stocks. Further agreement between our work and Friesen's occurred when we irradiated pupae of ages 24-36 hours and obtained practically no phenocopies, in keeping with Friesen's results when he treated flies with 2000 r. units at 120-144 hours after egg laying. Friesen suggests that the effects produced by temperature and X-ray treatments differ because of the relative specificity of different characters, certain phenotypes being produced by particular treatments: for example, dumpy wings by heat, bristle suppression by X ray. However, we have produced dumpy wings by X radiation, and Child, Blanc, and Plough (1940) have recorded a large percentage of flies with missing bristles in their work with temperature. We assume that the differences are due to different effective periods of the two agents, and possibly to differences in kind and extent of reaction.

Specific X-ray sensitive periods are suggested in our work and shown clearly by Friesen. What, then, is the relation of time of X-ray action to definite stages of development and to the temperature effective periods? Robertson (1936) reported that the trichogenic cells first appear at 30 hours of pupal age. Apparently X radiation, in producing reduction of macrochaetes, affected embryological processes, which are morphologically visible, only indirectly, as the treatment was applied at 0-12 hours of pupal age, before the time of bristle formation. Child, Blanc, and Plough (1940) stated that sublethal temperature may produce phenocopies either by directly affecting morphological processes or by influencing chemicophysiological reactions before the time of morphological differentiation. Gratziansky (1939) observed that a structure may be affected by X-ray treatment before the time of formation of the structure; he obtained notched wings by treating flies in the second day of larval life, before the time of formation of the wing disc. Gratziansky refers to this as an example of the *aftereffect* of X rays. He suggests that if the fly is



irradiated prior to the sensitive period of a particular phenocopy, cytophysiological "traces" may persist until the occurrence of the sensitive period and thus produce an effect. Lobashov and Solodovnikov (1939) also speak of the aftereffect of X rays.

According to this concept, as the gap between time of treatment and sensitive period becomes shorter, more traces are left, consequently producing a greater effect. Certain objections, however, may be raised to this explanation, depending upon what the term "sensitive period" signifies. If it means a period of morphological differentiation, how can one explain nonsensitive periods separating treatment-effective times and the affected stages of differentiation? Bristle reduction is effected by treatment some hours ahead of formation of the trichogenic cells, but is not brought about by treatment in the intervening period. If, on the other hand, the sensitive period is a time when the relations of chemicophysiological processes are altered, then any concept of passive storage would seem to be unnecessary.

Most of the phenocopies produced by irradiation of prepupae have been duplicated by temperature treatment, but the sensitive periods were different. Child (1935) found a temperature-effective period for reduction of macrochaetes in the third larval instar, but Child, Blanc, and Plough (1940) failed to find such a period in the prepupal instar, the period of sensitivity to X rays. These are not the only instars sensitive to bristle reduction. Ives (1940), with more severe temperature treatment, markedly increased the number of temperature-effective periods involved. Such a multiplicity of sensitive periods is in contradistinction to a single period of visible ontogeny. This is explained by the current concept of alteration of relative rates of reaction at different points in the complicated series of interrelated physicochemical processes which leads to visible differentiation.

## SUMMARY

### PART I

(1) Prepupae of *Drosophila melanogaster* were exposed to X radiation for periods of  $\frac{1}{4}$  to  $4\frac{1}{2}$  minutes (at approximately 940 r. units per minute), and the imagines were examined for phenocopic effects.

(2) With increasing exposure, the percentage of flies showing phenocopies increases. This relationship is graphically represented by an s curve, with a linear relationship from 235 to 2115 r. units. At exposures of 3290 or more r. units, all flies show phenocopies.

(3) The increase in number of phenocopies per fly is in linear proportion to increase in radiation from 1410 up to 3290 r. units, the highest dosage for which significant data are available.

(4) The curve for decrease in viability with increased radiation is a typical mortality curve, a sharp drop in viability occurring from 2350–3290 r. units.

(5) Explanation of any physical action of X radiation in producing phenocopies must account for the increases in rate of production.

## PART II

(1) Phenocopies produced by irradiation of *Drosophila* prepupae with different dosages of X rays are compared with respect to minimal effective dosage, rate of increase in number of flies affected with increase in dosage, and sex differences. Particular attention is paid to reduction of macrochaetes.

(2) Comparison of the reductions, both in size and number, of ocellar, anterior and posterior dorsocentral, and anterior and posterior scutellar bristles indicates close similarity with respect to early occurrence of effects, equality of response in males and females, and parallelism of average frequency of reaction of right and left sides.

(3) The anterior and posterior dorsocentrals show a distinctly different rate of increase in number affected as the dosage increases, whereas the anterior and posterior scutellars are markedly similar in this regard.

(4) The anterior-posterior and left-right associations between pairs of bristles with respect to phenocopic response throughout the range of treatment may be expressed as straight-line relationships.

(5) The data show a reasonable correspondence with those of Friesen.

(6) Differences in phenotypic effects of temperature and X radiation are explained on the basis of different times and modes of action of the two agents.

(7) The concept of aftereffect of X rays is rejected.

(8) The action of X rays in producing phenocopies is discussed on the basis of the current rate theory of development.

## STATISTICAL APPENDIX

In a statistical analysis of the degree of relationship between any two bristles, a number of possibilities present themselves. The bristles may be completely independent of each other in reaction with all treatments; they may have the same degree of interdependence throughout; or they may show an increasing degree of association with increasing dosage. In short, there is a series of hypotheses to be tested. We are greatly indebted to Dr. Jerzy Neyman of the Statistical Laboratory of the Department of Mathematics at the University of California, who very kindly undertook to develop for us an application of his method for the testing of composite hypotheses (Neyman, 1929). Responsibility for any errors in the presentation is ours.

Let us consider two bristles which we may designate as  $A$  and  $B$  when they are normal and as  $a$  and  $b$  when they are affected. If  $P_t(b/A)$  is used to denote the probability that  $B$  is affected when  $A$  is unaffected in flies subjected to  $t$  quarters of a minute of radiation, and correspondingly  $P_t(b/a)$ , the probability that both are affected, then the situation of mutual independence between the two bristles is given by

$$P_t(b/a) = P_t(b/A) \quad (1)$$

If this hypothesis of mutual independence, which we may call  $H_0$ , is found to be unlikely on the basis of a  $\chi^2$  test of the observational data, we may proceed to the next hypothesis,  $H_1$ , that the two probabilities exhibit a constant difference  $u_0$ . In other words,

$$P_t(b/a) = P_t(b/A) + u_0 \quad (2)$$

If this in turn is found to be unlikely, we may consider a third hypothesis  $H_2$  that

$$P_t(b/a) = P_t(b/A) + u_0 + u_1 t \quad (3)$$

This is the hypothesis of an increasing degree of association with increasing dosage. Similarly, we may continue this process, testing the hypothesis  $H_r$  that

$$P_t(b/a) = P_t(b/A) + u_0 + u_1 t + \dots + u_{r-1} t^{r-1} \quad (4)$$

The letter  $t$  may denote the actual number of quarter minutes of irradiation or the positive or negative excess of that number over a fixed limit  $t_0$ , which in our calculations equals 7 quarter minutes ( $1\frac{3}{4}'$ ).

The  $\chi^2$  test for (4) is based on the minimum of the expression

$$\chi^2 = \sum_i \left( \frac{[k_i - n_i P_t(b/A)]^2}{k_i(n_i - k_i)} n_i + \frac{(l_i - m_i [P_t(b/A) + u_0 + u_1 t + \dots + u_{r-1} t^{r-1}])^2}{l_i(m_i - l_i)} m_i \right)$$

with number of degrees of freedom

$$df_r = s - r \quad (5)$$

in which  $k_i$  = number of flies with bristle  $A$  normal and bristle  $B$  affected, i.e., with  $A$  and  $b$ ,

$n_i$  = number with  $A$ ,

$l_i$  = number with  $a$  and  $b$ ,

$m_i$  = number with  $a$ ,

and

$s$  = number of doses, with  $t$  representing the dose of radiation.

For the sake of simplicity, we may use the following notations in the tests of the particular hypotheses (1), (2), and (3). Let

$$q_i = \frac{k_i}{n_i}$$

$$Q_i = \frac{l_i}{m_i}$$

$$\bar{w}_i = \frac{n_i^2 m_i^2}{n_i^3 l_i (m_i - l_i) + m_i^3 k_i (n_i - k_i)}$$

Further,  $A = \sum w_i$ ,  $B = \sum l w_i$ ,  $C = \sum t^2 w_i$ ,  $D = \sum (Q_i - q_i) w_i$ , and  $E = \sum t(Q_i - q_i) w_i$ .

Then the  $\chi^2$  test for  $H_0$ , the hypothesis of independence, is

$$\chi_0^2 = \sum_i w_i (q_i - Q_i)^2 \text{ with } df_0 = s \quad (6)$$

For  $H_1$ , the hypothesis of a constant difference,

$$\chi^2 = \chi_0^2 + \bar{u}_0 \sum_i w_i (q_i - Q_i) \text{ with } df = s - 1 \quad (7)$$

or

$$\chi^2 = \chi_0^2 - \bar{u}_0 D \quad (7a)$$

in which  $\bar{u}_0 = D/A$ , with a standard error taken as equal to

$$1/\sqrt{A} \quad (8)$$

For  $H_2$ , the hypothesis of a linear increase with increase of treatment,

$$\chi_2^2 = \chi_0^2 - \bar{u}_0 D - \bar{u}_1 E \text{ with } df_2 = s - 2 \quad (9)$$

in which the estimates of the constants are

$$\bar{u}_0 = \frac{CD - BE}{AC - B^2} \quad (10)$$

and

$$\bar{u}_1 = \frac{AE - BD}{AC - B^2} \quad (11)$$

with standard errors taken as equal to

$$\frac{\bar{C}}{AC - B^2} \text{ and } \sqrt{AC - B^2}$$

respectively.

These tests are valid when  $n_i$ ,  $k_i$ ,  $m_i$ , and  $l_i$  are "large," i.e., when  $n_i$  and  $m_i$  are greater than 50 and  $k_i$  and  $l_i$  are at least 5. Unfortunately, our data attain these proportions only after two or more minutes of treatment. We have therefore made two sets of calculations, the first row figures for each pair of bristles being based on the data for treatments of 2-3 minutes, i.e.,  $t = 1$  to 5, and the second row indicating preliminary results based on the total data. We have fixed  $t_0$  as equal to  $1\frac{1}{4}$  minutes for the sake of ease in handling the data.

The first column of figures in the tables shows that the data do not fit the hypothesis of independence, since most of the values for  $\chi_0^2$  are beyond the 1-per cent level of significance and seldom are the values within the 5-per cent level. The values for  $\chi_1^2$ , testing hypothesis  $H_1$ , are less positive. Most of the figures do not militate against the hypothesis, but a few (in italics) are beyond the 1-per cent or 5-per cent levels of significance. Tentatively rejecting as a general rule the hypothesis that a constant difference exists between

TABLE 1  
CHI-SQUARE VALUES AND CONSTANTS FOR FEMALES  
(Chi-square values in italics are beyond the 5-per cent level of significance)

Pair of bristles tested	$\chi_0^2$	df <sub>0</sub>	$\bar{u}_0 \pm S. E.$	$\chi_1^2$	df <sub>1</sub>	$\bar{u}_0 \pm S. E.$	$\bar{u}_1 \pm S. E.$	$\chi_2^2$	df <sub>2</sub>
oc/adc.....	<i>23.801</i>	5	.086 $\pm$ .0180	.025	4	.075 $\pm$ .0490	.004 $\pm$ .0145	.427	3
	<i>32.989</i>	10	.032 $\pm$ .0132	<i>27.133</i>	9	.018 $\pm$ .0137	.018 $\pm$ .0047	12.469	8
adc/pdc.....	<i>11.768</i>	5	-.108 $\pm$ .0374	3.344	4	-.080 $\pm$ .0748	-.011 $\pm$ .0248	3.095	3
	13.110	9	-.076 $\pm$ .0295	6.422	8	-.058 $\pm$ .0316	-.019 $\pm$ .0117	3.712	7
pdc/asc.....	<i>22.400</i>	5	.067 $\pm$ .0162	5.650	4	.044 $\pm$ .0447	.007 $\pm$ .0131	5.509	3
	<i>34.212</i>	8	.075 $\pm$ .0149	8.862	7	.081 $\pm$ .0219	-.003 $\pm$ .0068	9.063	6
asc/psc.....	<i>54.112</i>	5	.252 $\pm$ .0346	1.444	4	.231 $\pm$ .0762	.007 $\pm$ .0239	1.612	3
	<i>88.404</i>	10	.271 $\pm$ .0302	10.627	9	.293 $\pm$ .0374	-.013 $\pm$ .0123	9.734	8
oc/asc.....	<i>74.569</i>	5	.199 $\pm$ .0243	5.914	4	.074 $\pm$ .0592	.042 $\pm$ .0184	1.243	3
	<i>82.331</i>	10	.172 $\pm$ .0209	14.047	9	.129 $\pm$ .0249	.026 $\pm$ .0082	3.766	8
ocs.....	<i>50.451</i>	5	.240 $\pm$ .0357	5.331	4	.147 $\pm$ .0819	.032 $\pm$ .0256	3.902	3
	<i>73.408</i>	10	.247 $\pm$ .0316	9.435	9	.246 $\pm$ .0374	.001 $\pm$ .0127	9.242	8
adcs.....	<i>48.063</i>	5	.201 $\pm$ .0302	4.044	4	.088 $\pm$ .0790	.037 $\pm$ .0240	1.707	3
	<i>59.940</i>	11	.188 $\pm$ .0258	7.676	10	.165 $\pm$ .0305	.014 $\pm$ .0097	5.558	9
pdcs.....	<i>11.099</i>	4	.140 $\pm$ .0458	1.719	3	.191 $\pm$ .0872	-.018 $\pm$ .0267	1.200	2
	<i>15.588</i>	7	.154 $\pm$ .0428	2.652	6	.198 $\pm$ .0592	-.020 $\pm$ .0186	1.416	5
ascs.....	<i>102.991</i>	5	.339 $\pm$ .0332	2.308	4	.267 $\pm$ .0812	.024 $\pm$ .0248	1.492	3
	<i>106.400</i>	8	.265 $\pm$ .0288	<i>21.600</i>	8	.189 $\pm$ .0332	.048 $\pm$ .0111	2.672	7
pscs.....	<i>56.689</i>	5	.343 $\pm$ .0475	4.553	4	.238 $\pm$ .1039	.036 $\pm$ .0316	3.449	3
	<i>61.331</i>	8	.298 $\pm$ .0418	10.373	7	.230 $\pm$ .0500	.040 $\pm$ .0162	4.241	6

TABLE 2  
CHI-SQUARE VALUES AND CONSTANTS FOR MALES  
(Chi-square values in italics are beyond the 5-per cent level of significance)

Pair of bristles tested	$\chi_0^2$	df <sub>0</sub>	$\bar{u}_0 \pm$ S. E.	$\chi_1^2$	df <sub>1</sub>	$\bar{u}_0 \pm$ S. E.	$\bar{u}_1 \pm$ S. E.	$\chi_2^2$	df <sub>2</sub>
oc/adc.....	1.160	5	.002 $\pm$ .0200	1.148	4	.001 $\pm$ .0145	.001 $\pm$ .0055	1.136	3
	4.023	10	.004 $\pm$ .0143	3.947	9	.005 $\pm$ .0480	— .002 $\pm$ .0164	3.814	8
adc/pdc.....	5.953	5	.021 $\pm$ .0354	5.596	4	.033 $\pm$ .0707	.005 $\pm$ .0249	5.562	3
	10.587	11	.034 $\pm$ .0302	9.329	10	.042 $\pm$ .0332	— .007 $\pm$ .0115	8.935	9
pdc/asc.....	17.039	5	.047 $\pm$ .0189	<i>10.835</i>	4	— .056 $\pm$ .0400	<i>.049</i> $\pm$ .0144	1.770	3
	<i>22.696</i>	8	.054 $\pm$ .0174	13.138	7	.032 $\pm$ .0242	.012 $\pm$ .0092	11.452	6
asc/psc.....	<i>42.960</i>	5	.194 $\pm$ .0358	<i>13.472</i>	4	.193 $\pm$ .0400	.001 $\pm$ .0146	<i>13.145</i>	3
	<i>45.177</i>	9	.172 $\pm$ .0318	<i>15.937</i>	8	.152 $\pm$ .0762	.012 $\pm$ .0270	<i>15.969</i>	7
ocs.....	<i>28.222</i>	5	.176 $\pm$ .0362	4.638	4	.192 $\pm$ .0775	— .006 $\pm$ .0257	4.588	3
	<i>30.373</i>	8	.161 $\pm$ .0324	5.745	7	.146 $\pm$ .0424	.008 $\pm$ .0148	5.488	6
ades.....	<i>42.084</i>	5	.203 $\pm$ .0349	8.366	4	.007 $\pm$ .0787	.075 $\pm$ .0270	.627	3
	<i>63.344</i>	11	.213 $\pm$ .0289	9.881	10	.206 $\pm$ .0316	.006 $\pm$ .0110	9.610	9
pdcs.....	<i>13.461</i>	5	.166 $\pm$ .0477	1.343	4	.212 $\pm$ .1020	— .018 $\pm$ .0360	1.081	3
	<i>15.567</i>	7	.170 $\pm$ .0455	1.626	6	.198 $\pm$ .0735	— .013 $\pm$ .0262	1.424	5
ascs.....	<i>34.580</i>	5	.225 $\pm$ .0391	1.505	4	.285 $\pm$ .0927	— .021 $\pm$ .0292	1.064	3
	<i>41.413</i>	9	.208 $\pm$ .0342	4.389	8	.196 $\pm$ .0424	.007 $\pm$ .0141	4.082	7
pscs.....	<i>27.090</i>	5	.220 $\pm$ .0492	7.009	4	.171 $\pm$ .0927	.021 $\pm$ .0332	6.638	3
	<i>28.787</i>	6	.223 $\pm$ .0477	6.933	5	.189 $\pm$ .0837	.016 $\pm$ .0315	6.585	4

any two bristles, and proceeding to the third hypothesis,  $H_3$ , we find that  $\chi^2$  values are for the most part small. Only the  $\chi^2_{df_2}$  for the relationship between anterior and posterior scutellars in the males is significantly large, beyond the 5-per cent level for  $df_2 = 7$  and beyond the 1-per cent level for  $df_2 = 3$ . One such value in eighteen may well occur by chance. Unfortunately, most of the  $\bar{u}_1$  values are not significant, and whether they would become significant with larger numbers of flies is not known.

We are faced then with two hypotheses, both of which satisfy the available data and both of which have interesting biological implications. We are dealing with the results of treatment of prepupae which may differ in age by as much as twelve hours. Sensitivity to treatment, for bristle effects may well be greater in one part of this range than in others. The degree of sensitivity and the extent of its period of occurrence will determine the value of  $u_0$ . If the various bristles have different sensitivity periods sufficiently removed from each other,  $u_0$  will be a negative quantity. A change in correlation with a change in dosage may be accounted for by assuming that the dosage has an effect on the shape and spread of the sensitivity curve, i.e., on the degree of sensitivity and on the extent of the sensitivity period.

We must confess that, for lack of adequate data, the results of our mathematical treatment have not been highly illuminating. We include this appendix chiefly for the value of the statistical method it presents.

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THE EFFECT OF X RADIATION UPON BRISTLE  
PATTERN IN DROSOPHILA MELANOGASTER

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# THE EFFECT OF X RADIATION UPON BRISTLE PATTERN IN *DROSOPHILA MELANOGASTER*

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AS A POSSIBLE APPROACH to the study of bristle pattern in *Drosophila melanogaster*, the use of the production of phenocopies by X radiation is suggested by Blanc and Braun (this volume), who noted a frequent absence of bristles when

TABLE 1  
BRISTLE SUPPRESSION IN CANTON

Bristle affected	♂ ♂				♀ ♀			
	Number of half flies affected		Percentage of half flies affected		Number of half flies affected		Percentage of half flies affected	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
orb a.....	0	26	0	16.7	0	27	0	23.3
m.....	0	11	0	7.1	0	0	0	0
p.....	0	21	0	13.5	0	17	0	16.4
oc.....	0	21	0	13.5	0	13	0	11.2
v 1.....	0	0	0	0	0	5	0	4.3
2.....	0	11	0	7.6	0	13	0	11.2
pv.....	0	19	0	12.2	0	17	0	14.7
hu 1.....	0	26	0	16.7	0	21	0	18.1
2.....	0	36	0	23.1	0	23	0	19.8
ps.....	1	29	0.5	19.1	0	21	0	18.1
npl 1.....	0	12	0	7.7	0	17	0	14.7
2.....	0	10	0	6.4	0	7	0	6.0
sa 1.....	0	19	0	12.2	0	10	0	8.6
2.....	0	8	0	5.1	0	7	0	6.0
dc 1.....	0	13	0	8.3	0	22	0	19.0
2.....	0	3	0	1.9	0	1	0	0.9
pa 1.....	0	10	0	6.4	0	2	0	1.7
2.....	0	26	0	16.7	0	20	0	17.2
sc 1.....	0	15	0	9.6	0	10	0	8.6
2.....	0	6	0	3.8	0	1	0	0.9
Total half flies in group.....	184	156			174	116		

*Drosophila* prepupae were irradiated. The question was next raised whether stocks which were genetically determined for the absence of one or more bristles in a definite percentage of instances would react to this treatment in a manner different from wild stocks. A brief report of this work has been given previously (Blanc and Villee, 1941). The present paper is a further report of the results of irradiation of stocks containing various scute alleles and their compounds. Assistance rendered by personnel of Work Projects Administration, Official Project 165-1-08-73, Unit C, is gratefully acknowledged.

## MATERIALS AND METHODS

The mutant stocks used were scute, yellow scute-5, scute-6 apricot, and scute-10 apricot. In addition we treated heterozygous scute flies and the compounds resulting from the crosses  $y\ sc^5\ ♀ \times sc\ ♂$ ,  $y\ sc^5\ ♀ \times sc^6\ w^a\ ♂$ , and  $sc^6\ w^a\ ♀ \times sc\ ♂$ . The wild stock Canton-S was used for purposes of comparison.

TABLE 2  
BRISTLE SUPPRESSION IN SCUTE

Bristle affected	♂ ♂				♀ ♀			
	Number of half flies affected		Percentage of half flies affected		Number of half flies affected		Percentage of half flies affected	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
orb a.....	219	165	99.6	99.5	256	158	97.0	98.7
m.....	191	159	86.9	95.9	164	112	62.1	70.0
p.....	0	30	0	18.9	0	17	0	10.0
oc.....	220	162	100	97.7	262	155	99.2	96.8
v 1.....	1	9	0.5	5.4	0	7	0	4.3
2.....	0	27	0	16.3	0	20	0	12.5
pv.....	209	157	95.0	94.7	238	146	97.6	91.2
hu 1.....	0	25	0	15.1	0	23	0	14.3
2.....	0	44	0	26.5	1	31	0.3	19.3
ps.....	0	30	0	18.1	0	24	0	15.0
npl 1.....	216	162	98.2	97.7	184	141	69.7	88.1
2.....	0	7	0	4.2	2	4	0.7	2.5
sa 1.....	1	33	0.5	19.9	0	35	0	21.8
2.....	0	9	0	5.4	0	3	0	1.8
oc 1.....	0	25	0	15.1	1	32	0.3	20.0
2.....	0	2	0	1.2	0	1	0	.6
pa 1.....	0	13	0	7.8	0	14	0	8.7
2.....	0	46	0	27.7	0	26	0	22.5
sc 1.....	215	161	97.7	97.1	201	118	76.1	73.5
2.....	218	165	99.1	99.5	254	148	96.2	92.5
Total half flies in group.....	220	166			264	160		

Larvae were raised in standard half-pint culture bottles under optimal food conditions, and kept at room temperature; pupae and prepupae were removed daily. Prepupae were selected on the basis of the presence of longitudinal tracheae and irradiated within 20 minutes with 2115 units ( $2\frac{1}{4}$  minutes at 940 r units per minute) of X radiation. The remaining pupae were kept as controls. Both experimental and control pupae and prepupae were left to complete their development on moist filter paper in Petri dishes at 25°C. As the imagines hatched they were counted and recorded for bristle effects. The X-ray apparatus and technique used were identical with those described previously by Blanc and Braun (this volume). The  $2\frac{1}{4}$ -minute radiation treatment was used, because this had been found to be the period of greatest phenocopy production with low lethality.

The many types of phenocopies found, some comparable to those noted by Blanc and Braun, included: all stages of bristle reduction; hooked, bent, forked, fused, doubled, and extra bristles; curled, curved, spread, jaunty, crumpled, wavy, spiral, and opaque wings; abnormal abdomen, abnormal legs, and rough eyes. Although effects were noticed on bristles other than those recorded, we have confined ourselves to those bristles in which differences

TABLE 3  
BRISTLE SUPPRESSION IN  $\gamma$  SC<sup>5</sup>

Bristle affected	$\sigma^{\circ} \sigma^{\circ}$				$\eta^{\circ} \eta^{\circ}$			
	Number of half flies affected		Percentage of half flies affected		Number of half flies affected		Percentage of half flies affected	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
orb a.....	0	34	0	22.4	0	16	0	13.7
n.....	0	6	0	3.9	0	2	0	1.7
p.....	0	13	0	8.6	0	9	0	7.7
oc .....	0	16	0	10.5	0	18	0	15.5
v 1.....	0	4	0	2.6	0	3	0	2.5
2.....	0	9	0	5.9	0	3	0	2.5
pv .....	1	32	0.4	21.1	0	25	0	21.5
hu 1.....	0	38	0	25.0	0	34	0	29.3
2.....	0	29	0	19.1	0	29	0	24.9
ps .....	0	25	0	16.5	0	15	0	12.9
npl 1.....	0	13	0	8.5	0	9	0	7.7
2.....	0	3	0	2.0	0	2	0	1.7
sa 1.....	0	24	0	15.8	0	15	0	12.9
2.....	0	4	0	2.6	0	2	0	1.7
dc 1.....	0	20	0	13.2	0	23	0	19.8
2.....	0	1	0	.6	1	5	0.4	4.3
pa 1.....	0	5	0	3.3	0	5	0	4.3
2.....	0	16	0	10.5	0	23	0	19.8
sc 1.....	33	57	14.4	37.5	6	26	2.5	22.4
2.....	45	60	19.7	39.5	9	19	3.8	16.3
Total half flies in group.....	228	152			236	116		

are most easily and accurately checked. In the tables we consider as "affected" only bristles that were completely suppressed, leaving no trace of bristle or ringpore.

The results are in tables 1-9. Tables 1-5 give the number and percentage of bristle effects in control and irradiated flies. The totals are based on half flies, which entails the assumption of genetic homogeneity within each of the several stocks. The percentages of bristle suppression in control and experimental flies (tables 6 and 7) afford a comparison of the effects of radiation on different bristles in the several stocks. Those bristles in each stock which are suppressed in the control flies are marked so that a comparison can be made of

the effects of radiation on bristles already affected and those not affected in the controls. Dashes are used in the tables wherever 85 per cent or more of the bristles were suppressed in the controls, since the significance of the values obtained is questionable and hardly comparable to the rest of the data. Chi-square tests of significance were made, and results which exceed the 5-per cent level of probability are in boldface type.

TABLE 4  
BRISTLE SUPPRESSION IN  $sc^b w^a$

Bristle affected	$\sigma^b \sigma^b$				$\eta \eta$			
	Number of half flies affected		Percentage of half flies affected		Number of half flies affected		Percentage of half flies affected	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
orb a.....	198	318	98.0	100.0	177	363	92.2	97.6
m.....	198	313	98.0	98.3	170	344	91.5	92.5
p.....	4	48	2.0	15.1	13	105	7.0	28.2
oc.....	200	309	99.0	97.0	182	370	97.9	99.5
v 1.....	0	16	0	5.0	0	20	0	5.4
2.....	0	31	0	9.7	0	46	0	12.4
pv.....	186	287	92.1	90.0	157	347	84.5	93.3
hu 1.....	0	49	0	15.4	1	57	0.5	15.3
2.....	1	43	0.5	138.0	1	55	0.5	14.8
ps.....	0	48	0	15.1	1	56	0.5	15.1
npl 1.....	158	280	78.2	87.9	88	186	47.3	50.0
2.....	0	5	0	1.6	0	12	0	3.2
sa 1.....	0	29	0	9.1	0	51	0	13.7
2.....	2	11	1.0	3.5	0	15	0	4.0
dc 1.....	0	35	0	11.0	0	43	0	11.6
2.....	0	5	0	1.6	0	4	0	1.1
pa 1.....	9	43	4.5	13.5	2	35	1.1	9.4
2.....	2	51	1.0	16.0	0	70	0	18.8
sc 1.....	0	20	0	6.3	0	33	0	8.9
2.....	0	7	0	2.2	0	7	0	1.9
Total half flies in group.....	202	318			186	372		

Table 8 was derived from tables 6 and 7 by averaging the difference between the control and treated values for the several stocks, excluding those values which differed markedly. Listed as significant variations are those in which bristle suppression was disproportionately high compared with other stocks. The variations in boldface and lightface type are those in which corresponding bristles in the control stocks are suppressed and unsuppressed, respectively.

Table 9 affords a comparison of the effects of genetic factors other than those in the X chromosome upon bristle pattern, comparing the effects of radiation upon males from the  $sc^b$  stock and upon  $sc^b$  males from  $sc^b \eta \times sc \sigma^b$  and  $sc^b \eta \times sc^b \sigma^b$  crosses.

TABLE 5  
BRISTLE SUPPRESSION IN  $sc^{10} w^a$

Bristle affected	♂ ♂				♀ ♀			
	Number of half flies affected		Percentage of half flies affected		Number of half flies affected		Percentage of half flies affected	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
orb a.....	0	7	0	12.9	0	1	0	6.3
m.....	0	3	0	5.5	0	0	0	0
p.....	0	10	0	18.5	0	2	0	12.5
oc .....	0	9	0	16.6	1	1	0.7	6.3
v 1.....	0	4	0	7.4	0	1	0	6.3
2.....	0	3	0	5.5	1	1	0.7	6.3
pv .....	0	5	0	9.2	0	7	0	43.8
hu 1.....	6	11	4.7	20.3	10	2	7.5	12.5
2.....	7	19	5.5	35.1	9	2	6.7	12.5
ps .....	0	10	0	18.5	0	1	0	6.3
npl 1.....	0	6	0	11.1	0	2	0	12.5
2.....	0	2	0	3.7	0	0	0	0
sa 1.....	3	10	2.3	18.5	0	8	0	50.0
2.....	5	9	3.9	16.6	7	6	5.2	37.5
dc 1.....	122	52	96.7	96.2	113	16	83.4	100.0
2.....	126	54	100.0	100.0	134	16	100.0	100.0
pa 1.....	0	4	0	7.4	0	0	0	0
2.....	0	9	0	16.5	0	5	0	31.2
sc 1.....	0	2	0	3.7	0	0	0	0
2.....	0	1	0	1.8	0	0	0	0
Total half flies in group.....	126	54			134	16		

## DISCUSSION

### SEX DIFFERENCES

Where there are differences in suppression in the controls, the males show more than the females (tables 1-5). The mean suppression by X radiation is about the same in the two sexes: for males, 10.5 per cent; for females, 9.68 per cent. Among bristles showing significant variation (table 8) there are a few sex differences which will be discussed later.

### BRISTLE DIFFERENCES

Comparison of the mean differences of the various bristles shows clearly that the reaction to radiation is not always similar. The median orbital, first vertical, second notopleural, second supraalar, second dorsocentral, and second scutellar bristles show a slight suppression of bristles after treatment, whereas the anterior orbital, second humeral, presutural, and second postalar bristles



show the strongest suppression. No pattern of suppression is discernible; bristles showing high and low suppression seem distributed at random. There is no difference between the suppression of head and body bristles; their averages are comparable.

#### STOCK DIFFERENCES

Tables 6 and 7 show a number of bristles which react very similarly to treatment in all the stocks. Some bristles react similarly; a number show some de-

TABLE 6  
DIFFERENCES BETWEEN TREATED AND CONTROL FEMALES  
(in Per Cent)

Bristle affected	+	sc	sc <sup>5</sup>	sc <sup>6</sup>	sc <sup>10</sup>	+/sc	sc <sup>5</sup> /sc	sc <sup>6</sup> /sc <sup>5</sup>	sc <sup>6</sup> /sc
orb a ..	23.3	..	13.7	..	6.3	14.4	18.9	17.3	..
m..	0	<b>7.9<sup>o</sup></b>	1.7	..	0	0	0	3.8	6.7 <sup>o</sup>
p ..	16.4	10.6	7.7	<b>21.2<sup>a</sup></b>	12.5	15.1	15.9	15.4	16.4
oc ...	11.2	..	15.5	..	5.6	7.5	10.6	18.3	<b>54.2<sup>a</sup></b>
v 1 ..	4.3	4.3	2.5	5.4	6.3	2.1	2.3	4.8	7.7
2 ..	4.2	12.5	2.5	12.4	6.3	4.8	6.1	2.9	15.4
pv ...	14.7	..	21.5	..	<b>43.8</b>	13.0	17.4	<b>47.1<sup>o</sup></b>	..
hu 1 ..	18.1	14.3	<b>29.3</b>	14.8	5.0 <sup>a</sup>	18.5	19.7	12.5	7.7
2 ..	19.8	19.0	24.9	14.3	5.8 <sup>a</sup>	15.8	23.5	9.6	0
ps ...	18.1	15.0	12.9	14.6	6.3	8.2	18.9	16.3	15.4
npl 1 ..	14.7	<b>18.4<sup>o</sup></b>	7.7	2.7 <sup>b</sup>	12.5	5.4	3.3	13.5	11.9 <sup>a</sup>
2 ..	6.0	1.8	1.7	3.2	0	1.4	2.3	1.0	0
sa 1 ..	8.6	<b>21.8</b>	12.9	13.7	<b>50.0</b>	11.0	12.1	14.4	3.8
2 ..	6.0	1.8	1.7	4.0	<b>32.3<sup>a</sup></b>	0	2.3	2.9	0
dc 1 ..	19.0	19.7	19.8	11.6	..	6.2	8.3	10.6	7.7
2 ..	0.9	0.6	3.9	1.1	..	0	1.5	2.9	0
pa 1 ..	1.7	8.7	4.3	8.3	0	3.4	7.8	5.8	0
2 ..	17.2	<b>22.5</b>	19.8	18.8	31.2	15.1	15.9	10.6	19.2
sc 1 ..	8.6	..	<b>19.9<sup>a</sup></b>	8.9	0	6.9	<b>11.7<sup>a</sup></b>	8.6	11.5
2 ..	0.9	..	<b>12.5<sup>a</sup></b>	1.9	..	0.7	<b>23.1<sup>b</sup></b>	5.8	0

<sup>a</sup> 0-25 per cent suppressed in controls.

<sup>b</sup> 26-50 per cent suppressed in controls.

<sup>o</sup> 51-85 per cent suppressed in controls.

gree of variation; others react very differently in the several stocks. Thus the first vertical, second notopleural, second dorsocentral, and first postalar bristles show little variation in the amount of suppression in all the stocks, whereas the presutural and a number of other bristles show a wider range of variation without any single value differing significantly from the others. These small differences may be due to the result of minor genetic differences in the stocks or to errors of random sampling. Those instances in which one member of the series showed a striking increase over the average are recorded in table 8 and will be discussed individually.

Table 8 shows that of the 22 cases of extreme increase, 14 (shown in bold-face) had some amount of bristle suppression in the control flies, due to genetic reasons, whereas the other eight did not. This indicates that those bristles

which are predisposed genetically toward suppression are affected to a greater degree by treatment than are those which are normal under control conditions. Applying the concept of a threshold for bristle suppression or formation to these data, we may assume that those bristles which are not present in 100 per cent of flies under control conditions are very close to this threshold and therefore are affected to a greater extent by radiation than those bristles which are present in 100 per cent of flies under control conditions. The latter may be assumed to be farther removed from this threshold and therefore more con-

TABLE 7  
DIFFERENCE BETWEEN TREATED AND CONTROL MALES  
(in Per Cent)

Bristle affected	+	sc	sc <sup>a</sup>	sc <sup>b</sup>	sc <sup>10</sup>
orb a .....	16.7	..	22.4	..	12.9
b .....	7.1	..	3.9	..	5.5
p .....	13.5	18.1	8.6	13.1	18.5
oc .....	13.5	..	10.5	..	16.6
v 1 .....	0	4.9	2.6	5.0	7.4
2 .....	7.1	16.3	5.9	9.7	5.5
pv 1 .....	12.2	..	20.7	..	9.2
hu 1 .....	16.7	15.1	25.0	15.4	15.6 <sup>a</sup>
2 .....	23.1	26.5	19.1	13.3	29.6 <sup>a</sup>
ps .....	18.6	18.1	16.5	15.1	18.5
npl 1 .....	7.7	..	8.5	9.7 <sup>b</sup>	11.1
2 .....	6.4	4.2	2.0	1.6	3.7
sa 1 .....	12.2	19.9	15.8	9.1	16.2
2 .....	5.1	5.4	2.6	2.5	12.7 <sup>a</sup>
dc 1 .....	8.3	15.1	13.2	11.0	..
2 .....	1.9	1.2	0.6	1.6	..
pa 1 .....	6.4	7.8	3.3	9.0 <sup>a</sup>	7.4
2 .....	16.7	27.7	10.5	15.0	16.5
sc 1 .....	9.6	..	23.1 <sup>a</sup>	6.3	3.7
2 .....	3.8	..	19.8 <sup>a</sup>	2.2	1.8

<sup>a</sup> 0-25 per cent suppressed in controls.

<sup>b</sup> 51-85 per cent suppressed in controls.

stant in appearance. The eight instances mentioned above may be bristles lying just above this threshold and therefore bristles which, though not affected under control conditions, are affected greatly by radiation.

Most of these eight instances can be shown to be of rather questionable validity when they are analyzed further. The value for the second vertical in the scute male is high compared to the male, but not the female average; the difference is only slightly significant for the male, and insignificant for the female, and thus may be an error of random sampling. Similar considerations hold for the second postalar bristles of scute males and for the postvertical bristles of scute-5 males.

The number of the treated scute-10 females was low, making the resulting percentages of bristles affected of doubtful accuracy unless corroborated by

evidence from the male values, which were high enough to be valid. Thus, the values for the postvertical and the anterior supra-alar bristles may not be valid, whereas in the posterior supra-alars, which are also affected in the males, the difference probably is valid.

TABLE 8  
MEAN DIFFERENCES BETWEEN TREATED AND CONTROL FLIES AND  
SIGNIFICANT VARIATIONS FROM THE MEAN

Bristles affected	Mean differences in per cent between treated and control ♀♀	Significant variations in ♀♀	Mean differences in per cent between treated and control ♂♂	Significant variations in ♂♂	Bristles affected in controls but not showing significant variations
orb a	14.4		17.3		
m	0.6	sc = 7.9	5.5		
p	11.8	sc <sup>5</sup> = 21.2	14.4		
oc	10.8	sc <sup>5</sup> /sc = 54.2	13.5		
v 1	4.6		3.2		
2	10.6		7.1	sc = 16.3	
pv	18.1	$\left\{ \begin{array}{l} sc^{10} = 43.8 \\ sc^5/sc = 47.1 \end{array} \right\}$	10.7	sc <sup>5</sup> = 20.7	
hu 1	13.1	sc <sup>5</sup> = 29.3	15.7	sc <sup>5</sup> = 25.0	sc <sup>10</sup> ♀♀    sc <sup>10</sup> ♂♂
2	16.8		22.3		sc <sup>10</sup> ♀♀    sc <sup>10</sup> ♂♂
ps	13.4		17.4		
npl 1	9.4	sc = 18.4	9.1	sc <sup>5</sup> = 9.7	sc <sup>5</sup> ♀♀
2	2.5		3.6		
sa 1	11.7	$\left\{ \begin{array}{l} sc = 21.8 \\ sc^{10} = 50.0 \end{array} \right\}$	14.6		
2	3.4	sc <sup>10</sup> = 32.3	2.8	sc <sup>10</sup> = 12.7	
dc 1	17.5		11.9		
2	1.6		1.3		
pa 1	4.6		6.8		sc <sup>5</sup> ♂♂
2	21.9		14.7	sc = 27.7	
sc 1	5.8	$\left\{ \begin{array}{l} sc^5 = 19.9 \\ sc^5/sc = 11.7 \end{array} \right\}$	6.5	sc <sup>5</sup> = 23.1	
2	0.9	$\left\{ \begin{array}{l} sc^5 = 12.5 \\ sc^5/sc = 23.1 \end{array} \right\}$	2.6	sc <sup>5</sup> = 19.8	
Mean	9.68		10.05		

The value for the first supra-alar bristle of scute females is given as significant in table 8; nevertheless, the corresponding male value is also the highest of its category, yet not significantly above the average. Thus no definite conclusion can be reached with respect to the presence or absence of a significant effect of treatment on this bristle. A similar consideration may apply to both male and female scute-5 first humerals, although the evidence here points more clearly to a small but definite increase.

In contrast, all those instances are valid in which an extreme increase was noted in bristles already affected in the controls. The actual percentage of increase is always somewhat larger than that recorded because some of the

bristles are already removed in the control flies and the treatment therefore has a smaller percentage of bristles upon which to work. In some instances this consideration will raise the value only a few per cent, whereas in others, as in the first notopleural of scute-6 males, the actual increase is about five times 9.7 because almost 80 per cent of the bristles are already missing in the control flies. No values can be given for scute males for the median orbital and the first notopleural bristles, since almost 100 per cent of the bristles are already removed in the control flies.

TABLE 9  
BRISTLE ABSENCE IN NONIRRADIATED SCUTE-5 MALES  
(in Per Cent)

Bristle	$sc^5 \text{♀} \times sc^5 \text{♂}$	$sc^5 \text{♀} \times sc \text{♂}$	$sc^5 \text{♀} \times sc^5 \text{♂}$
	$\text{♂} \text{♂}$	$\text{♂} \text{♂}$	$\text{♂} \text{♂}$
a sc.....	14.4 $\pm$ 2.33	32.4 $\pm$ 5.00	45.6 $\pm$ 4.69
p sc.....	19.7 $\pm$ 2.63	50.0 $\pm$ 5.39	84.2 $\pm$ 3.46

There are a few instances listed in the last column of table 8 in which particular bristles were affected in the controls and in the experimentals to an equal degree. The scute-6 male first postalar value, although not extreme, is larger than that of any other in its category; an error of random sampling may have hidden a significant increase. This is true also for the male scute-10 value for the second humeral bristle. Again, the number of female scute-10 experimental flies was small and the validity of the values of these female humerals is questionable.

Genetic factors other than the scute alleles themselves may be involved in this experiment. This is suggested by a comparison of results obtained from three different lines of scute-5 males. Table 9 gives control (nonirradiated) values for the scutellar bristles of males obtained from crosses of  $sc^5 \text{♀} \times sc^5 \text{♂}$ ,  $sc^5 \text{♀} \times sc \text{♂}$ , and  $sc^5 \text{♀} \times sc^5 \text{♂}$ . The X chromosome in all flies is derived from the same source, the scute-5 stock. The striking differences obtained must therefore be ascribed to the autosomal reconstitutions brought about by the crosses, indicating that autosomal modifiers of the scute action are present in at least two of the stocks concerned.

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THE EFFECT OF CHANGES IN TIME OF  
DEVELOPMENT ON THE PHENOTYPE  
OF MUTANTS OF DROSOPHILA  
MELANOGASTER

BY  
WERNER BRAUN



# THE EFFECT OF CHANGES IN TIME OF DEVELOPMENT ON THE PHENOTYPE OF MUTANTS OF *DROSOPHILA* *MELANOGASTER*

BY  
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## I. THE EFFECT ON NOTCHED WING CHARACTERS

RECENTLY PUBLISHED (Braun, 1939) were the first results of experiments dealing with the effect of prolonged development on the amount of wing destruction of  $vg^{no}$  and  $ct^a$  strains. In these experiments the amount of destruction of

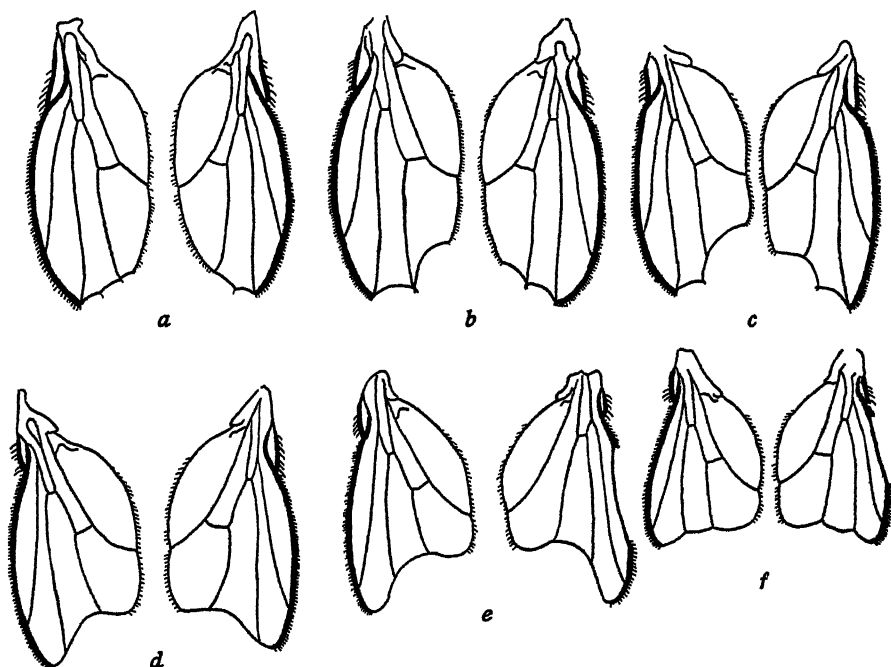


Fig. 1. Degrees of notch effect of  $vg^{no}$  wings of flies hatching on successive days, time of development prolonged by transfer to peptone at 24-48-hour larval age: (a) at 10 days, normal notching; (b) 11 days, slight; (c) 11 days, moderate; (d) 12 days, strong; (e) 13 days, very strong; (f) 14 days,  $ct$ -like. All  $\times 21$ .

wing area had been demonstrated to increase with the time of development (fig. 1). Flies of a  $vg^{no}$  or  $ct^a$  stock, which normally show only a slight terminal notch on the wing, were raised on peptone food ("partial starvation") during certain periods of their larval stages, or went without food for 3 days ("complete starvation"), and their time of development was thus delayed up to 17



days.\* The few flies that hatched 10 days after egg laying, the usual time of development, showed the normal amount of wing-area destruction. The flies that hatched after 12 days showed more destruction over the control flies, and those which hatched after 15 days showed such an extreme amount of destruction as is normally observed only in higher alleles of the *vg*- or *ct*-series. The results were clear, although only a few flies were examined, and the age of larvae at the time of transfer to peptone food or foodless conditions was not measured exactly. Some observations indicated that the pattern of destruction varied with the degree of starvation. The results of a more extended analysis, and of a few recent experiments with food and temperature control are described in this paper. Services rendered by the personnel of Work Projects Administration, Official Projects 465-03-3-192, 165-1-08-73, Unit C-1, are gratefully acknowledged.

### MATERIALS AND METHODS

The method adopted for these experiments was first used by Beadle and co-workers (1938) and Khouvine, Ephrussi, and Chevais (1938), although they did not use it primarily for the purpose of prolonging development. Larvae were partly starved by transfer to peptone food (2 cc. of a mixture of 50 cc. Ringer's solution, 1.5 g. agar-agar, 2.5 g. glucose + 1 cc. of a 10-per cent Bacto-peptone solution in each vial). For complete starvation, larvae were transferred to Petri dishes containing filter paper soaked in Ringer's solution, kept there 1-3 days, and returned to normal food. In some experiments the larvae were not returned to normal food, and pupated in the Petri dishes. The age of the larvae was determined by allowing flies to lay eggs for a limited time only. At a given age the larvae were transferred, to finish their development on peptone food. Unless otherwise stated, the experiments were carried out at 25° C. A stock of *vg*<sup>20</sup>, a low allele of *vg*, which has been inbred for many years and shows a uniform type of slight notching under normal conditions, was used for most of the experiments described here. An inbred stock of *vg* was used for some of the experiments combining partial starvation with higher temperature. The hatched flies were preserved in 70-per cent alcohol, and their wings, subsequently mounted on slides, were drawn with the help of a microprojector. The analysis is based on a total of 959 flies.

### PARTIAL STARVATION

Two differently timed sets of experiments were performed with peptone food. In the first set accurately timed larvae were transferred to the vials containing peptone food. They were offspring of parents whose period of egg laying had been restricted to 2 hours. The results of this experiment are assembled in table 1, experiments 1-8. When larvae 23-92 hours old were transferred to peptone food, both the time of development and the destruction of wing area

\* In order to signify different modes of starvation the terms partial starvation (i.e. mildly starved, ill-fed), intermediate starvation (i.e. well starved, lowest food level) and complete starvation (i.e. severely starved, foodless) have been used throughout this paper. These terms should not be taken in the literal sense but rather as an easy way to distinguish between the different degrees of actual starvation.

increased. The time of development varied, and was most prolonged when larvae were transferred at 70–72 hours. Development was least prolonged after transfer at 23–25 and 90–92 hours. However, time of development of the larvae transferred at 23–25 hours may very well have been delayed during early larval stages, the deficiency being made up afterward. This is probable if the maximum effect observed in the first flies to emerge is considered. Table 1 shows clearly that the earlier the larvae are transferred to peptone food, the greater is the effect on notching. Of the flies transferred as 23–25-hour larvae,

TABLE 1

INCREASE IN DEGREE OF NOTCH EFFECT OF *vg<sup>ao</sup>* FLIES WITH PROLONGED DEVELOPMENT RESULTING FROM PEPTONE FOOD

Hours of larval age at transfer to peptone	Days of development and degree of notch effect*								
	9	10	11	12	13	14	15	16	17
1) 23–25.....		1–2–3	2–3–4	3–4					
2) 33–35 .....		0	0	1–2	2–3				
3) 46–48.....			0–1–2	1–2	2				
4) 48.....			0	0–1	1–2	3			
5) 49–51.....				1–2–3	3–4				
6) 55–57.....			0–1–2	1–2	3				
7) 72 .....			0	1–2	2–3	3	4	5	
8) 90–92.....		0	0–1	0–1					
9) 20–44.....			1–2	2–3	3–4				
10) 24–48.....			0–1	2–3	4	4	5	6	7
10a) 19–47.....			2–3		3–5	5			
11) 44–68.....		0	0–1	0–1	1–2–3	2–3	3	4–5	
12) 48–70.....			0–1–2	1–2	3	3–4	4	3–4	
13) 50–80.....			0	0	0	1–2			
14) 68–92.....		0–1	0	0–1					

\* Explanation of degree of notch effect.:  
 0 Normal (as in *vg<sup>ao</sup>*)      2 Moderate  
 1 Slight                      3 Strong

4 Very strong  
 5 Ct-like

6 Strongly cut  
 7 Extremely cut

the first individuals emerging after 10 days show an increased amount of notching. The same degree of notching is observed only after a 2-day delay in emerging in flies transferred after 33–35 hours. The degree of notching exhibited by 23–25-hour flies emerging after 12 days of development is equaled by the 72–74-hour flies only if the development of the latter is delayed 5 days. The effect on flies transferred as late as 90–92 hours of larval is slight, probably because these old larvae pupate soon after transfer—even earlier than normal—and are underfed for only a short time.

This set of experiments shows that after partial starvation the degree of wing notching of the flies first to emerge increases with the length of time in which the larvae are underfed. No definite sensitive period for starvation can therefore be established under conditions of partial starvation.

The second set of experiments differed from the first only in that the age of the larvae was not as precisely checked at the time of transfer. The mothers of

the larvae were allowed to lay eggs over a period of 24 hours. The results are essentially like those observed after more exact timing of larval age, except for a greater variation, to be expected under such conditions (table 1, experiments 9-14). No clear relation, however, existed between the age of larvae at transfer to peptone food and prolongation of time of development.

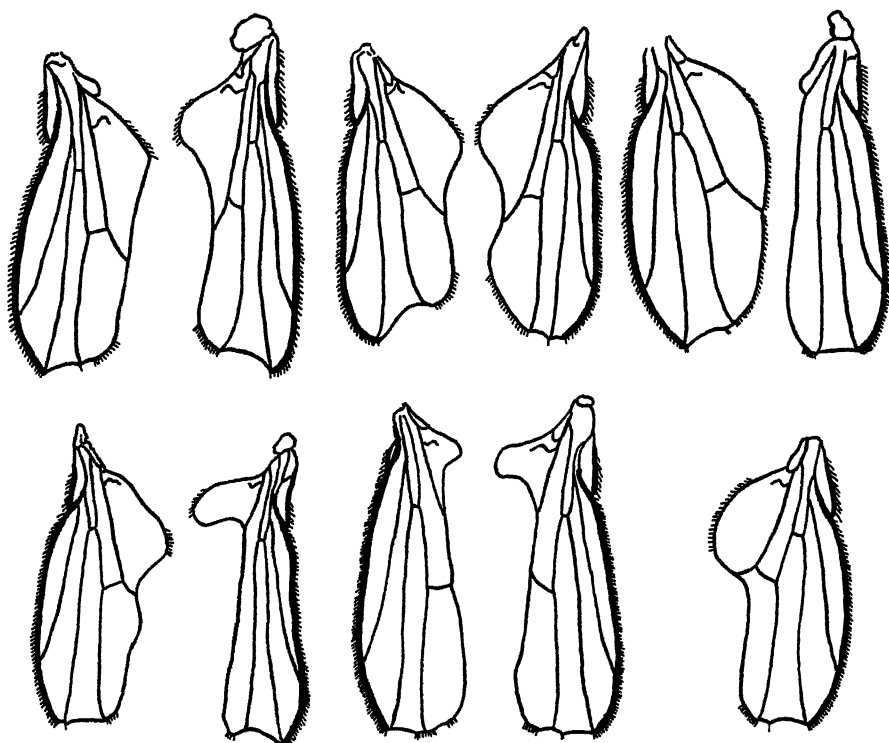


Fig. 2. The *P* and *Ma* types of destruction encountered after complete starvation of 22-74-hour  $vg^{no}$  larvae.  $\times 22$ .

In almost all partially starved flies the destruction increased from the tip of the wing and toward the proximal end. Marginal destruction was observed in only 3 males, from larvae transferred at 90-92 hours, and 3 other males, which showed the highest degree of destruction,  $vg^{no}$  (5) type.

### COMPLETE STARVATION

When complete starvation was applied to developing  $vg^{no}$  flies, the results were quite different in several respects. The most obvious difference was the pattern of destruction. In contrast to the results of partial starvation, little effect was observed at the distal end of the wing. Moreover, completely different patterns of destruction were encountered. Sometimes the destruction affected the posterior margin of the wing, resulting in a modification, *P*, resembling the mutants *Beaded* and *Beadex*. In extreme specimens this modification resembles wing patterns in Goldschmidt's so-called *Ma* stock, which

combines the vg mutant and certain dominigenes (see Gardner, in this volume). At other times, the destruction affected the anterior margin, modification A, also resembling the mutant Beadex, and sometimes the mutant Beaded. An antler-like form of destruction, also produced, was earlier known as a modification of certain vg stocks. These three types of destruction were produced as specific modifications dependent on the larval age at which starvation began.

Larvae which were removed from food at larval stages of 43-74 hours, and returned to normal food after 3 days, developed into flies, many (up to 100 per cent) of which showed wings of the *P* or *Ma* type (table 2 and fig. 2).

TABLE 2

MODIFYING EFFECT ON VG<sup>20</sup> WINGS OF 3-DAY STARVATION AT DIFFERENT LARVAL AGES

Hours of larval age at removal from normal food	Days of development	Number hatched	Per cent showing modification	Type of modification
1) 43-47.....	12-14	7	14	<i>P</i> - <i>Ma</i>
2) 46-48.....	11-13	14	0	.....
3) 44-46.....	12-14	7	14	<i>P</i> - <i>Ma</i>
4) 44-48.....	13-16	15	6	<i>P</i> - <i>Ma</i>
5) 48-50.....	14-15	3	0	.....
6) 66-68.....	13-17	37	49	<i>P</i> - <i>Ma</i>
7) 66-68.....	13-14	7	71	<i>P</i> - <i>Ma</i>
8) 70-72.....	13-16	18	44	<i>P</i> - <i>Ma</i>
9) 72-74.....	14-17	21	38	<i>P</i> - <i>Ma</i>
10) 46-70.....	12-14	17	59	Antlerlike
11) 47-71.....	13	3	100	<i>P</i> - <i>Ma</i>

The mortality was extremely high when larvae under 43 hours old were starved for 3 days. Therefore, in a second set of experiments (table 3, experiments 12-18), larvae were starved for only 2 days, starting at stages varying from 23 to 72 hours. These larvae developed into flies 3-75 per cent of which exhibited wing destruction of the *P* or *Ma* type. In one experiment 22-24-hour larvae were removed from food for one day (table 3, experiment 19). Even after this short foodless period, at an early stage, 8 per cent of the flies showed an increased destruction of the *P* or *Ma* type.

The antlerlike pattern of destruction was encountered in one experiment (no. 10 of table 2, and fig. 3), in which 46-70-hour larvae were removed from all food for 3 days. Some older larvae, of the same experiment were not returned to food, developed into flies which showed no antlerlike destruction, but developed a form typical of larvae which pupate during starvation. Presumably, then, starvation begun at an age of 46-70 hours produces this antlerlike pattern. Additional tests are needed to show whether an antlerlike pattern can be produced when starvation begins at an age between 50 and 60 hours. This pattern may be the result of a simultaneous destruction of the *Ma* type and a destructive process starting from the distal end of the wing.

A different type of destruction, scalloping of the anterior margin, was encountered if complete starvation started after 70 hours of larval age and the

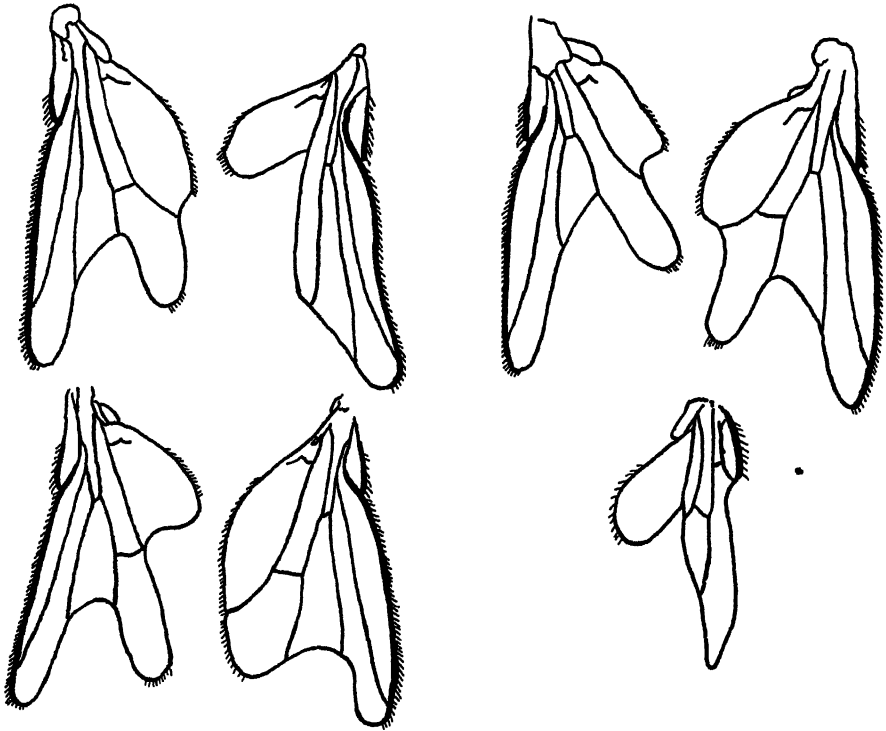


Fig. 3. The antlerlike pattern of destruction from experiment no. 10.  $\times 27$ .

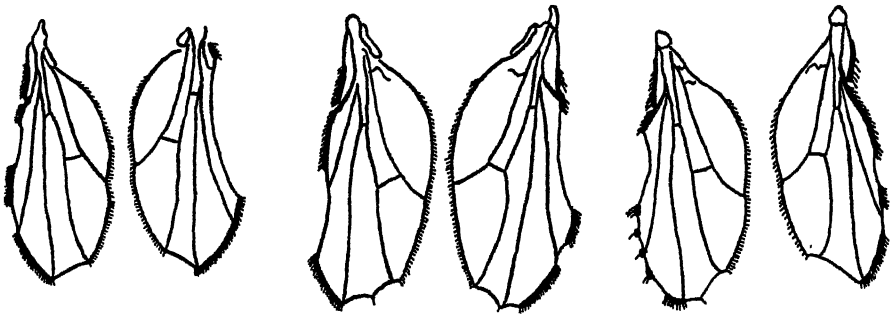


Fig. 4. The A type of destruction encountered after complete starvation of  $vg^{ao}$  larvae 70 hours or more of age.  $\times 25$ .

larvae pupated without further food (table 4 and fig. 4). Beadle (1938) reports that complete starvation starting prior to 70 hours results in delayed pupation; after 70 hours, in accelerated pupation. Goldschmidt observed in *Lymantria dispar* that moulting and pupation may be hastened by starvation. Acceleration of pupation under such conditions was also observed in my experiments. The flies which hatched from such pupae showed only the A type, never the P or Ma type. The specificity of the production of the A type after acceleration in the late larval stages was easily controlled. The majority of larvae used in these particular experiments were about 70 hours old. Larvae younger than 70

hours at onset of starvation had not yet pupated after 3 days and were returned to normal food. Flies hatching from these larvae exhibited the *P*, *Ma*, or antlerlike pattern (experiments no. 10, 11, and 18 in tables 2 and 3). A number of the older larvae of the same experiments, however, apparently those older than 70 hours when starvation started, had already pupated when the

TABLE 3

MODIFYING EFFECT ON  $vg^{ao}$  WINGS OF 1-2-DAY STARVATION AT DIFFERENT LARVAL AGES

Hours of larval age at removal from normal food	Days of starvation	Days of development	Number hatched	Per cent showing modification	Type of modification
12) 46-48.....	2	12	4	75	<i>P-Ma</i>
13) 46-48.....	2	11-13	21	75	<i>P-Ma</i>
14) 67-69.....	2	12-15	34	43	<i>P-Ma</i>
15) 23-47.....	2	11-13	7	29	<i>P-Ma</i>
16) 24-48 small larvae....	2	11-15	37	3	<i>P-Ma</i>
17) 24-48 large larvae....	2	11-12	15	40	<i>P-Ma</i>
18) 48-72.....	2	11-13	8	37	<i>P-Ma</i>
19) 22-24.....	1	10-23	60	8	<i>P-Ma</i>

TABLE 4

MODIFYING EFFECT ON  $vg^{ao}$  WINGS OF STARVATION AFFECTING STAGES AFTER 70 HOURS OF LARVAL DEVELOPMENT

Hours of larval age at removal from normal food	Days of development	Number hatched	Per cent showing modification	Type of modification
20) 68-70.....	9	3	100	<i>A</i>
21) 90-92.....	9-11	8	50	<i>A</i>
10a) 46-70.....	9-11	7	43	<i>A</i>
11a) 47-71.....	8-10	25	84	<i>A</i>
18a) 48-72.....	10	6	67	<i>A</i>

younger ones were transferred back to normal food. Among the flies hatching from these accelerated pupae 43-100 per cent showed destruction of *A* type only (experiments no. 10a, 11a, and 18a in table 4).

The data compiled in table 2 indicate the existence of a sensitive period for increased destruction after complete starvation. The percentage of flies showing a modified type of destruction varies greatly, the average being 8.15 per cent if starvation starts at 43-48 hours of larval age, and 50.5 per cent at 66-74 hours; the percentage increases toward the 70-hour period if larvae are completely starved for 3 days. Such behavior, however, is not indicated after 2 days of starvation (table 3). Here, the percentage of flies showing increased destruction varied greatly and irregularly for the different periods at which starvation started. In only two experiments, 16 and 17, which were performed with larvae from the same culture, does the percentage of modified flies increase with the delay in onset of starvation. For experiment 16 small, younger

larvae were selected from a timed culture. After 2 days of starvation they developed into flies 3 per cent of which showed modified destruction. For experiment 17 the large larvae of the same culture were used and starved for 2 days; these older larvae developed into flies 40 per cent of which showed a modified pattern of destruction. The percentage of flies with modified patterns of destruction was not regular among the flies that developed from larvae removed from food after the 70-hour period.

TABLE 5  
CHANGES IN NOTCH EFFECT ON *vg*<sup>20</sup> FLIES INDUCED BY PEPTONE FOOD  
AND HIGHER TEMPERATURE

Hours of larval age at onset	New type of food	New temperature applied, deg. C.	Days of development and degree of notch effect*								Number hatched
			8	9	10	11	12	13	14	15	
50-74	peptone	25			0-1	1-2	3-4-5	4			32
50-74	normal	30	0	normal-0	normal-0						51
50-74	peptone	30		0-1	0-1	1-2	2-3				44
20-44	peptone	25				1-2	2-3	3-4			36
20-44	peptone	30		0-1	2-3	2-3					38
44-68	peptone	25			0	0-1	0-1	1-2-3	2-3	3	42
44-68	peptone	30		1	1	0-1-2	3				40
68-92	peptone	25			0-1	0	0-1				26
68-92	peptone	30		0-1	1	1	1				32

\* See footnote, table 1.

The amount of destruction of wing area, measured for 40 wings of the *P* and *Ma* type, did not appear significantly related to larval age at onset of starvation; this age seems, rather, to determine the specific patterns of destruction.

The effect of complete starvation on the time of development can be demonstrated in our experiments. The data of table 2 indicate that after 3 days of complete starvation starting at larval ages of 43-72 hours, the time of development increases from 1-4 days, in proportion to the age of the larvae at the time of removal from food. The flies hatch 1-2 days late if complete starvation is applied for 2 days only (table 3). No delay in hatching can be observed after 1 day of complete starvation starting at the larval ages of 22-24 hours. The duration of larval instars would have to be checked in order to find out whether the time of development is prolonged at some early stage in development and then compensated for by accelerated development in a later stage. The data of table 4, finally, indicate that flies hatch 1-2 days earlier if complete starvation starts after the 70-hour period.

## PARTIAL STARVATION AT HIGHER TEMPERATURE

EXPERIMENTS WITH  $vg^{20}$ 

In a number of tests the larvae were raised on peptone food at 30° C. instead of 25° C. The results of these experiments, combining partial starvation with temperature effects, are presented in table 5. The larvae for the experiments of each age group originated from the same culture and were transferred at the

TABLE 6

CHANGES IN NOTCH EFFECT ON  $vg^{20}$  FLIES INDUCED BY INTERMEDIATE STARVATION

Medium no.	Hours of larval age at time of transfer	Days of development and degree of notch effect*						
		10	11	12	13	14	15	16
1	42-48	0	0-1	1				
1	61-67	..	0-1-2	0-1	0-1-2	2		
1	68-72	0	0-1 Some Bx					
1	46-61	1	1	1-2	1-2 Some Bx	1		
2	42-48		1	...	...	1-2		
2	61-67	0	0-1	1-2-3	1-2	1-2		
2	68-72	-2	0-1	1-2	1-2	2 Some Bx	1-2	
3	42-48	..	..	...	...	...	1	
3	61-67	1	0	0-2	2			
3	68-72	..	0-1	0-2 Some Bx	1-2	2	8	9
3	46-61			1-2	2	1		

\* Explanation of degree of notch effect:

0—Normal  
1—Slight  
2—Moderate3—Strong  
4—Very strong  
5—Ct-like

6—Strongly cut

7—Extremely cut

8—Ca. 60 per cent destroyed

9—Ca. 75 per cent destroyed

same day and hour, thus insuring similarity in environmental conditions with the exception of the difference in food or temperature applied. The experiments listed in the first three rows of table 5 show that, compared with the results at 25° C., the amount of notching *decreased* if larvae were raised in normal food at a temperature of 30° C. starting at 50-74 hours of larval age: 45 per cent of the flies had completely normal wings without any notches; 49 per cent had only one wing notched. When the larvae were raised in peptone



TABLE 7

CHANGES IN NOTCH EFFECT ON  $vg^{ao}$  FLIES INDUCED BY TEMPORARY STARVATION  
OF 42-48-HOUR LARVAE

Results in **boldface** type indicate larvae returned to normal food after 4 days

Medium no.	Days of development and degree of notch effect					
	10	11	12	13	14	15
2.....		1-2	1-2			
2.....			0	0	0	
3.....						1
3.....					0-1 (all Bd)	0
3.....					0	
3.....				0	0-1-2 almost all Bd	0 almost all Bd

food at 30° C., no difference in the notch effect was observed in the experiment started at 50-74 hours of larval age. However, in all the other experiments the amount of destruction *increased* significantly when the larvae were raised on peptone food at 30° C., this increase being greater than at 25° C.\*

Flies hatching from larvae partially starved at 30° C. show the same degree of destruction as at 25° C. when retarded 2 more days. For example, flies from 20-44-hour larvae transferred to peptone food and 30° C. show a destruction of type 2-3 when they hatch after 10 days of development, whereas a destruction of this intensity is reached only after a development of 12 days at 25° C.

Flies raised on peptone food start to hatch 1 day earlier (9 days after egg laying) at 30° C. than at 25° C., and 1 day later than on normal food at 30° C.

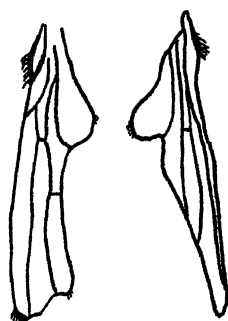


Fig. 5. Straplike wing representing the greatest increase in  $vg$  wing area after larvae have been raised on normal food at 30° C.  $\times 22$ .

#### EXPERIMENTS WITH $VG$

The effect of temperature on the development of  $vg$  wings has been investigated by a number of workers (Roberts, 1918; Harnly, 1930, 1932, 1933, 1936, 1940; Harnly and Harnly, 1935, 1936; Hersh and Ward, 1932; Stanley, 1931, 1935; Riedel, 1934), who observed that wing area increases with temperature. In many experiments, performed simultaneously with those on  $vg^{ao}$ , I detected this: *when  $vg$  larvae were raised on peptone food at 25° C. wing area did not increase; however, at 30° C. the wing area increased more than with normal*

\* The phrase "increase in wing area" is used here for descriptive purposes only; actually it is chiefly a decrease in wing destruction, as Goldschmidt has maintained in his work.

food at 30° C. (figs. 5 and 6). The larvae were transferred to peptone food and 30° C. at 27-74 hours, but data are too few to explain the effect of the different starting times for partial starvation and temperature increase. Clearly, however, the increase in wing area of flies raised on normal food at 30° C. reached its maximum in a straplike wing, which represents primarily an increase in the

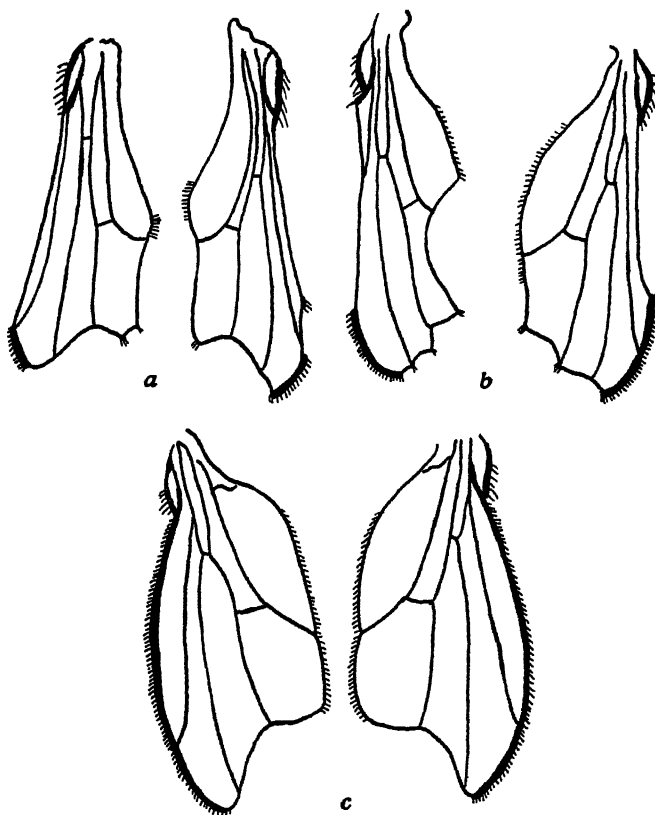


Fig. 6. Increase in wing area of *vg* flies hatching on successive days; larvae raised on peptone at 30° C.: (a and b) at 9 days; (c) at 10 days [*vg*<sup>no</sup> (3) type].  $\times 29$ .

length of the *vg* wing. With 30° C. and peptone food, the straplike wing increased in width, resulting in a *vg*<sup>no</sup> (3) type; flies hatching on the 9th and 10th day show an increase in wing area comparable to the maximum straplike increase of flies hatching from normal food at 30° C. Flies hatching on the 11th day from peptone food show the above mentioned increase in width of wing.

These preliminary experiments, combining temperature effect with partial starvation, indicate that *vg* and *vg*<sup>no</sup> show opposite effects: *vg*<sup>no</sup> flies from larvae on peptone food at 30° C. exhibit an increase in wing destruction; *vg* flies, a decrease.

## DISCUSSION

That the phenotypes of *vg* and its alleles can be changed by environmental and genetic factors, especially temperature and dominigenes, has been demonstrated by a number of workers (Roberts, 1918; Harnly, 1930, 1932, 1933, 1936, 1940; Harnly and Harnly, 1935, 1936; Hersh and Ward, 1932; Stanley, 1931, 1935; Riedel, 1934; Mohr, 1932; Goldschmidt, 1935, 1937; Green and Oliver, 1940). Mutants at the *vg* locus are seen to modify rates and duration of constructive and destructive processes during development. The advantage of starvation is that it so delays development that the delay can be easily recognized and measured. The effects of temperature and dominigenes on growth are more difficult to evaluate. Significantly, different patterns of destruction are produced if partial or complete starvation is applied during different larval ages, at least three distinct patterns appearing in these experiments: (1) a distoproximal destruction after partial starvation; (2) destruction starting from the posterior margin (*P* type); (3) destruction starting from the anterior margin (*A* type). Another type of destruction, the antlerlike pattern, needs more careful analysis.

The patterns might be determined by at least two processes: one determines the differentiation of the wing length, and is affected by partial starvation; another determines the differentiation of the width, and is affected by complete starvation. (This is perhaps comparable to Sinnott's size and shape genes.) The existence of at least two separate processes is substantiated by a few preliminary experiments, combining partial and complete starvation, in which the effects on the pattern of destruction appear additive. The complex of reactions determining wing length and wing width must differ because partial starvation never increases marginal destruction and complete starvation never increases a distoproximal destruction. Application of an "intermediate" starvation may show whether there is a definite threshold for the complete starvation effect, or whether a fluctuation of the effect on the pattern of destruction exists between complete and partial starvation.

To determine whether starvation actually prolongs development, or only retards it, *vg<sup>no</sup>* and the mutant giant were combined in a recent preliminary experiment. The mutation giant is in the first chromosome and produces giant flies by causing an extra larval instar. Flies showing *vg<sup>no</sup>* and giant did not show any increased wing destruction, although the time of development of giant flies is prolonged. This result indicates that the changes in destructive patterns caused by changes in time of development are not due to a *prolongation* of development but rather to a *retardation*. Interpretations which may explain all the phenomena observed will be postponed until these and some other experiments are finished. I may point out, however, that this work substantiates the assumption expressed before (Braun, 1939) that most notched wing patterns may be produced by the interaction of a destructive factor and the rate of development. The coördination and the different intensity of these factors, and the different stages in development at which they can act, may produce the many different notch patterns known in *Drosophila*. These pat-

terns can belong to one series of alleles or they can be completely different genetically (Bx, Bd, vg, etc.).

A critical period at 70 hours of larval development was established for complete starvation. The significance of the 70-hour period in starvation experiments has previously been demonstrated by Beadle (1938) for his experiments on eye pigmentation. The importance of the same 70-hour period in wing development suggests that this time of transgression from the second to the third larval instar is of general importance for the determination of developmental processes.

It was noted that *vg* and *vg<sup>no</sup>* show opposite effects when exposed to partial starvation and temperature change at larval stages. Wing destruction increased when *vg<sup>no</sup>* larvae were used, but decreased if *vg* larvae were raised on peptone food at 30° C. If higher temperature alone was applied, wing destruction decreased in *vg* as well as in *vg<sup>no</sup>*. Partial starvation alone increased wing destruction in *vg<sup>no</sup>*, but had no effect on *vg* raised in peptone food at 25° C. Child (1939) added Nipagin to the food of *vg* larvae, thus producing partial starvation, and described the decrease of wing destruction. However, all his experiments were performed at 28° C. In terms of my observations, his results demonstrate not only the effect of partial starvation, but also that of increased temperature.

How can the opposition of the effects produced when *vg* and *vg<sup>no</sup>* are raised under conditions of partial starvation and higher temperature be explained? Perhaps temperature applied with partial starvation intensifies a modifying process the direction of which has been determined by the effect of partial starvation. Because *vg* and *vg<sup>no</sup>* react in opposite directions, similar experiments with other *vg* alleles are planned to see if the different *vg* alleles show a gradated series of effects between increase and decrease of wing destruction. In other words, it will be determined whether some *vg* allele (or combinations of *vg* alleles) exhibits neither increase nor decrease of its normal type of wing destruction after a combined application of partial starvation and higher temperature.

## II. ADDITIONAL DATA ON THE PRODUCTION OF THE DIFFERENT TYPES OF SCALLOPED WING PATTERN

To PROVIDE different degrees of starvation in a new set of experiments, the following media were used:

$$1 \text{ cc. of } \left\{ \begin{array}{l} 1. \text{ 5-per cent peptone solution} + 2.5 \text{ glucose} \\ 2. \text{ 2-per cent peptone solution} + 2.5 \text{ glucose} \\ 3. \text{ 2-per cent peptone solution} \end{array} \right\} + 2 \text{ cc. of 3-per cent agar solution per vial.}$$

To each vial of these media 30 larvae from inbred *vg*<sup>no</sup> stock were transferred at ages of 42–48, 61–67, and 68–72 hours. In one set of experiments the larvae were allowed to finish development on these media (table 1). Flies raised on medium 2 were modified essentially the same as in the earlier experiment with 10-per cent peptone solution, except for the appearance of a few type *A* flies, formerly occurring only after complete starvation. When larvae were raised on medium 2, mortality of larvae transferred at 42–48 hours was increased. Notching exceeded only slightly that occurring with medium 1. One type *A* fly appeared. More flies of type *A* were produced when larvae were raised on medium 3. Mortality of larvae transferred to this at 42–48 hours was extremely high. The degree of notching of their wings did not increase significantly above that of flies raised on medium 1, except that a few flies, which hatched after 15–16 days from the 68–72 hour-group, showed more destruction than ever before observed in these experiments (classes 8 and 9, almost *vg*). In contrast to the results with 10-per cent peptone solution, flies from media 1–3 were much reduced in size.

Since the mortality of larvae transferred to medium 2 or 3 at 42–48 hours was so high, other larvae were transferred back to normal food after 4 days (table 2). Flies thus raised on medium 2 showed almost no increase in destruction; on medium 3 terminal notching increased little, but almost all flies showed type-*P* modification (destruction of posterior margin), without decrease of body size. This type was formerly produced only after complete starvation.

### DISCUSSION

Partial starvation always resulted in an increase of terminal destruction, whereas complete starvation seemed only to increase marginal destruction. To explain these results it was assumed that the different kinds of starvation affect separate processes of determination: (1) for the width of the wing, (2) for the length of the wing. Was there a definite threshold for the complete starvation effect or was there a fluctuation of the effect of complete and partial starvation? The increase of destruction after partial starvation was shown to follow a path, from distal to proximal, determined by the retardation of development rates and therefore by the duration of partial starvation. After complete starvation, however, two different patterns of destruction were encountered, depending upon the age at which larvae were starved—type *P* if complete starvation started before 70 hours; type *A* if started after 70 hours. Type *A* can also occur if an intermediate kind of starvation (1, 2, or 3) is

applied (table 1), but type *P* occurs only when larvae are thus starved until pupation takes place. Strangely enough, if larvae are removed from medium 3 and returned to normal food, type-*P* modification occurs.

Some conclusions can be drawn from these results: (1) Modification of types *A* and *P*, although both appear after complete starvation, have different thresholds. This is indicated by the production of type *A* after intermediate starvation, which, if applied in the same manner, does not suffice to produce type *P*. (2) Type *P* cannot be produced by a continuous retardation of development; it is produced when a strong retardation is suddenly interrupted by return to normal conditions. In the complete starvation experiments, also, the larvae always had to be returned to normal food, as development ceased otherwise. So long as only the contrasting results of complete and partial starvation were known, I assumed that the different retardation produced during the starvation period was responsible for the different modification. However, in the light of the new results type *P* may be assumed to result from the sudden change of rates produced after interruption of the retarding influences. Medium 3, when applied until pupation, produced only terminal destruction, or type *A*. When, however, larvae were returned to normal environment from this medium, type modification *P* was produced. This suggests that only the interruption of retarding influences can be responsible for this type of modification.

By experimental production of phenotypic changes similar to those resulting from genic action, the normal action of the "gene" may be investigated. The modifications produced in starvation of *vg*<sup>20</sup> stock occur normally in a number of *vg* alleles and in the *Bx* and *Bd* mutants. The mutant *Bd* particularly exhibits destruction of the type *P*. If the action of the *Bd* mutant were to alter a chain of processes at the same stage of development as does starvation, then the *Bd* action could be indicated by an interpretation similar to that given to the production of type *P* after temporary retardation of development. Accordingly, the mutant *Bd* acts primarily by briefly retarding a chain of reactions for a limited time only, with the subsequent change to normal or different rates being responsible for wing modification. Its action then consists of a strictly time-limited change of rates. According to my results, it would not be effective if it extended its retarding action over a long period of development. On the other hand, terminal and type-*A* destruction can be produced by prolonged retardation of developmental rates, during a relatively long time of development. It can hardly be argued that perhaps the sensitive period for the production of *Bd* patterns is more limited and shorter. Medium 3 has a retarding effect during the period when the developing wing is sensitive to changes toward type *P*, but no such wing destruction occurs unless the retarding influences cease and the larvae are returned to normal conditions.

No definite conclusions can be drawn about the nature of the processes affected by the retarding influences. These influences undoubtedly affect a great number of reactions, but our results give no clues of their nature.

TABLE 8  
THE EFFECT OF PROLONGED DEVELOPMENT ON PHENOTYPES OF SEVERAL Cy STOCKS AND THE MUTANT CU

Stock used	Larval age at time of transfer to peptone food (in hours)	Days of development and degree of curling <sup>a</sup>									
		10	11	12	13	14	15	16	17		
tkd/Cy al L <sub>4</sub> sp <sup>2</sup> .....	26-50	a: 8 ♀ 2♂	a: 5 ♀ 9♂	a: 3 ♀ c: 2♂	c: 1 ♀ 1♂			a: 1 ♀			
tkd/Cy al L <sub>4</sub> sp <sup>2</sup> .....	26-50	a: 2 ♀ 3♂	a: 11 ♀ 12♂	a: 4 ♀ 2♂ c: 1 ♀ 2♂	a: 1 ♀						
tkd/Cy al L <sub>4</sub> sp <sup>2</sup> .....	28-52	a: 3 ♀ 2♂	a: 6 ♀ 2♂	b: 2 ♀ 1♂	a: 2 ♀ 3♂ c: 1♂	a: 1♂ c: 1♂					
al dp d b e px sp/Cy sp <sup>2</sup> .....	24-48		a: 1 ♀ 1♂ b: 1 ♀ 2♂	b: 1 ♀ c: 1♂	a: 2 ♀ b: 3 ♀ c: 2 ♀ d: 1♂	b: 1 ♀					
al dp d b e px sp/Cy sp <sup>2</sup> .....	45-69		a: 14 ♀ 8♂ b: 7♂	a: 2 ♀ 4♂ b: 1 ♀ 3♂ c: 1 ♀ 4♂	b: 1 ♀	c: 1 ♀ d: 1♂	d: 1♂	a: 1 ♀ b: 2 ♀	1 ♀		
cu.....	29-53	a: 1 ♀	a: 13 ♀ 7♂	a: 2 ♀ 1♂ b: 1 ♀ 2♂ c: 2♂ d: 2 ♀ 2♂	a: 1♂						
new cu II.....	50-72	a: 9 ♀ 4♂	a: 1 ♀ 1♂ c: 2♂	a: 1 ♀ b: 1♂ c: 1♂	c: 1♂						

### III. THE EFFECT OF CERTAIN CURLY STOCKS, AND A DEMONSTRATION OF THE NONSPECIFIC EFFECT OF DOMINIGENES

IN EXTENDING starvation treatment to other mutants, larvae of different Curly (Cy) stocks were partially starved on peptone food and their time of development thus prolonged. Curly is a dominant character which produces wings that are strongly curled upward. It is associated with Inversion (2L) Cy, or In (2L) Cy and In (2R) Cy (Ward, 1923). After discovery that the Cy phenotype of certain stocks could be changed by prolonging development, these Cy stocks were combined with vg dominigenes in order to determine whether these dominigenes (Goldschmidt, 1935, 1937) have a specific effect on vg alleles only, or whether they can change the phenotype of all characters affected by changes of developmental rates during the period at which they act. Earlier work (Goldschmidt, 1937; Braun, 1939) indicated that these vg dominigenes act by changing developmental rates. Further results of the combinations of certain Cy stocks with these vg dominigene stocks are also reported herein.

The method of partial starvation was identical with the one previously described in detail (Beadle, 1939; Braun, 1939). At different stages larvae were transferred to vials containing peptone food (10-per cent peptone solution, agar-agar, and glucose) to finish their development.

#### EFFECT ON THE PHENOTYPE OF CERTAIN Cy STOCKS

Table 8 shows the effect of partial starvation on the following Cy stocks: tkd/Cy al L<sub>4</sub> sp<sup>2</sup>, and al dp d b c px sp/Cy, sp<sup>2</sup>. After prolonged development many Cy flies showed an uncurling of the Cy wing. The effect is greater in

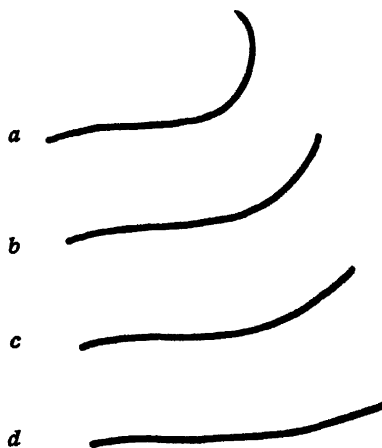


Fig. 7. Degree of curling (see table 8).

males than in females. The standard recessive mutant curled and a new recessive mutant curled in the second chromosome (unpublished) also exhibit uncurling of the wing after prolonged development (table 8). However, no effect was seen when partial starvation was applied to the following Cy stocks: (1) an/Cy, (2) d/Cy, (2L) dp<sup>2</sup> b pr, (3) S/Cy ES and (4) al dp b pr c px sp/Cy, pr. Flies from these stocks showed normally curled wings after prolonged development (table 2).

It should be noted that Cy flies from those stocks which exhibit a straightening out of the curly wing after prolonged development contain the recessive mutant sp<sup>2</sup> in the Cy chromosome. To show that the presence of speck in the Cy chromosome alters the physiological behavior of Cy, it was necessary to eliminate the speck gene from these Cy stocks and to test the reaction to partial starvation of these Cy stocks without speck. After many crosses, I finally separated the speck region of the chromosome from the inversion by



crossing over. Cy flies from this speck-free stock exhibited no uncurling of the wing after prolonged development (table 9).

### COMBINATIONS OF CERTAIN Cy STOCKS WITH TWO STOCKS CONTAINING *vg* DOMINIGENES

The results reported above suggested that it would be worthwhile to test the action of the *vg* dominigenes on those stocks of Cy in which Cy wings straighten after prolonged development. Dominigenes were first described by Gold-

TABLE 9

THE EFFECT OF PROLONGED DEVELOPMENT ON PHENOTYPES OF OTHER Cy STOCKS

Stock used	Larval age (in hours) after egg laying at time of transfer to peptone food	Days of development	Number hatched	Effect on Cy phenotype
an/Cy.....	20-44	10-13	16	None, except 1 13-day <i>c</i> ♂ <sup>a</sup>
an/Cy.....	24-48	11-13	19	None
an/Cy.....	48-72	10-12	14	None
an/Cy.....	48-72	10-13	23	None
d/Cy (2L) dp <sup>2</sup> b pr....	28-52	10-12	16	None
c/Cy (2L) dp <sup>2</sup> b pr.....	48-72	9-13	29	None, except 1 13-day <i>b</i> ♀
d/Cy (2L) dp <sup>2</sup> b pr.....	48-72	9-12	28	None
d/Cy (2L) dp <sup>2</sup> b pr....	48-72	9-12	21	None
d/Cy (2L) dp <sup>2</sup> b pr.....	48-72	9-13	28	None
S/Cy ES.....	48-72	10-15	47	None
S/Cy ES.....	48-72	10-13	37	None
S/Cy ES.....	48-72	10-12	41	None
al dp b pr c px sp/Cy, pr	0-48	10-15	30	None
al dp b pr c px sp/Cy, pr	22-46	10-11	17	None
al dp b pr c px sp/Cy, pr	48-69	11-16	15	None, except 1 16-day <i>b</i> ♀
Cy without sp <sup>2</sup> /S×Ore-R	60-72	10-14	22	None
Cy without sp <sup>2</sup> /S×Ore-R	48-60	10-16	25	None
Cy without sp <sup>2</sup> /Bl.....	44-68	10-13	18	None

<sup>a</sup> See footnote, table 8, for explanation of effect.

schmidt (1935, 1937), who discovered their action on *vg* alleles. They are modifying genes which shift the phenotype of a heterozygote in favor of dominance or increased dominance of one of the alleles. In the *vg* alleles they were said to act by shifting the threshold for the onset of a destructive process. In 1935 Goldschmidt observed that the phenotypic notched effect of heat shocks could be superimposed on the genetic notched effects of dominigene stocks. Gardner and Braun (Braun, 1939) later showed that a parallel of the action of the dominigenes could also be produced in heterozygous *vg* flies by raising their larvae on peptone food, thus prolonging their development. Therefore the action of the dominigenes might consist merely of a general prolongation of certain developmental stages. Indeed, Goldschmidt and Gardner (in

this volume) observe that dominigenes, as well as translocations and inversions, affect the mutants Bd, Bx, and vg. If the dominigenes act merely by a general prolongation of certain developmental stages, they could also affect the phenotype of Cy wings, provided that they can act during the critical period for uncurling.

Two different dominigene stocks (containing dominigenes and  $vg/+$ ) were used for these experiments: (1) the Doho stock, which always produces terminal notches in the wings of heterozygous  $vg$  flies, and (2) the stronger Doma stock, which usually produces large marginal wing destruction (Ma type) in heterozygous  $vg$  flies. Males from the dominigene stocks were crossed with  $aldp\ d\ b\ c\ px\ sp/Cy, sp^2$  females and the  $F_1$  females were backcrossed to males from the dominigene stocks.

A combination of  $Cy\ sp^2$  with dominigenes from the Doho stock showed no effect on the wings of  $Cy$  flies. However, in combinations of  $Cy\ sp^2$  with the dominigene from the Doma stock many  $Cy$  flies showed an uncurling (table 10). Again, the effect was greater in the males than in the females. The parents of these flies were left in the bottles for 8 days; therefore, unfortunately, no record exists as to the time of development of the flies with affected  $Cy$  wings. However, among the first  $Cy$  flies hatching 10 days after the start of the culture there were already many with affected wings, which may indicate that the dominigenes change developmental rates in a more specific manner, which does not affect the general time of development as much as when an environmental agent (starvation) is applied.

## DISCUSSION

Only in  $Cy$  stocks containing the recessive mutant  $speck^2$  ( $sp^2$ ) in the  $Cy$  chromosome does the  $Cy$  wing seem to uncurl after prolonged development. The presence of  $speck$  in the  $Cy$  chromosome thus alters the physiological behavior of  $Cy$ . The manner in which the Curly phenotype is produced is still uncertain. Some authors have maintained that curling is due to a change in pressure in the expanding adult wing, contending that the degree of curvature is affected by the temperature at which the adult wing expands. None of my several experiments revealed such an effect.  $Cy$  pupae transferred to  $30^\circ\text{C}$ . showed no effect of the higher temperature on the curvature. Another possible cause of curling is a growth differential in the upper and lower epithelia. However, study of cells in comparable portions of the upper and lower epithelia before folding revealed no differences in cell number or size. Curling may be produced by different chitinization, but this would be hard to prove experimentally. Interestingly, a pupal process can be influenced by changes during larval life.

From the results of the combinations of  $Cy\ sp^2$  with dominigenes we can conclude that the action of the dominigenes is not restricted to  $vg$  but is non-specific. The results presented here further indicate that the dominigenes act by prolonging developmental rates and, provided that they act at a critical time, should be able to influence all those characters which can be clearly affected by such prolongation. This general result is not influenced by

TABLE 10  
THE EFFECT OF DOMINIGENE "DOMA" ON THE PHENOTYPE OF CY WINGS<sup>a</sup>  
♀ MA I/, + Cy SP <sup>2</sup>/+ × ♂ MA I

Number of cross	+		Ma		vg		Cy d <sup>d</sup>		Cy b <sup>d</sup>		Cy c <sup>d</sup>		Cy d <sup>d</sup>		½ Cy	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
6001 <sup>b</sup> .....	34	24	12 (1)	12 (1)	..	..	15	8	8	9 (1)	6 (1)	7	2	6 (1)		
6002 <sup>b</sup> .....	33	32	4	11	..	..	16	12	5	7	4	2	1	3		
6003.....	12	12	2	13 (2)	11	13	26	20 (2)	5 (1)	6 (1)	3	5 (3+1)	2	11 (6+1)		2
6004.....	36	39	2	8 (1)	..	..	22	19	7	10 (2)	2	4	2	8		
6005.....	12	21	12	13	19	16	29	14	5	9 (1)	6	5 (2)	3 (1)	9 (4)		1
6006.....	8	15	2	7 (4)	18	13	30	21	12 (1)	17 (1+2)	5 (1)	4 (1)	....	3		
6007 <sup>c</sup> .....	2	2	8	10	7	4	20	4	2 (2)	9	8 (3)	4 (3)	3 (1)	3 (2)	..	2
6003-6007.	70	88	26	41 (7)	55	46	127	78 (2)	31 (4)	51 (1+6)	24 (4)	22 (6+4)	10 (2)	34 (12+1)	..	5

<sup>a</sup> Figures in **boldface type** indicate number of flies showing Ma; figures in *italics* indicate number of flies showing terminal notches.

<sup>b</sup> Not all flies were checked for Ma.

<sup>c</sup> Strong Ma.

<sup>d</sup> See footnote to table 8 for explanation of degree of curling.

the observation that the dominigenes from the Doho stock do not influence the Curly phenotype. Such a result could be expected from my earlier work on  $vg^{ao}$ , in which partial starvation resulted in a destruction which started from the distal end of the wing and increased towards the base of the wing, and complete starvation resulted in a destruction which always started from the posterior margin. It resembled the Ma type found in the Doma stock, where this particular pattern of destruction was produced by the influence of the dominigene on the heterozygous  $vg$ . The starvation work, therefore, showed that the mode of change in developmental rates produced by the different dominigenes must be different, just as the effect is different after partial or complete starvation. Now, one of these dominigene stocks can change the Curly phenotype in the same manner as prolongation of development by peptone food. This particular dominigene can, therefore, act at the critical time, and/or can control the development rate, which determines curling. The dominigenes in the Doho stock probably also change development rates, but at a time or in a manner that cannot affect the development of Cy wings.

If the reported results are considered in terms of dominance and recessiveness, it can be said that starvation and the presence of dominigenes weaken the dominance of the mutant Curly by changing development rates.

## SUMMARY

### PART I

(1) Complete or partial starvation of larvae appreciably changes time of development. The effect of this change upon notch pattern of  $vg^{ao}$  flies depends upon the degree of starvation and the larval age of its onset.

(2) Partial starvation at larval ages of 23–72 hours prolongs development and increases distoproximal notching, which increases with the time of starvation.

(3) Complete starvation for 1, 2, or 3 days at ages of 22–74 hours produces scalloping of the posterior margin of the wing. (An antlerlike pattern was produced in a special instance.) Complete starvation after 70-hour age accelerates pupation, always produces scalloping of the anterior margin of the wing, and effects a sensitive period for increased destruction.

(4) Results of a number of experiments employing starvation and temperature effects are presented.

(5) An explanation of the production of patterns of notching destruction is attempted, and results are cited which suggest that these changes result from retardation rather than prolongation of development.

### PART II

(1) Different degrees of starvation (intermediate types) reveal that modifications *A* and *P* have different thresholds.

(2) Type *P* is produced when a strong retardation of developmental rates is suddenly interrupted by return to normal conditions.

(3) An interpretation of the action of the mutant *Bd* is attempted on the basis of these results.

## PART III

(1) Prolonging of development caused by peptone food affects the phenotype of a number of Curly (Cy) stocks which contain the recessive mutant speck in the Cy chromosome in that the wings uncurl; Cy stocks without speck were not thus affected.

(2) Uncurling also occurred when certain Cy stocks were combined with vg dominigenes, which shows the nonspecific effect of the dominigenes and suggests that dominigenes can change the phenotype of all characters which can be affected by changes of developmental rates during the period at which they act.

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A FURTHER STUDY OF GENETIC MODIFICATION  
OF DOMINANCE, ESPECIALLY BY  
POSITION EFFECTS

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# A FURTHER STUDY OF GENETIC MODIFICATION OF DOMINANCE, ESPECIALLY BY POSITION EFFECTS

BY

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AS PART of a program of analysis of wing scalloping in *Drosophila* in its bearing upon fundamental conceptions of physiological genetics, this paper deals with the modification of dominance in the vestigial (vg) heterozygote by mutant loci and chromatin rearrangements in collaboration with the dominance modifiers (dominigenes) described by Goldschmidt (1935-37). Goldschmidt and Gardner (elsewhere in this volume), cite the literature on the subject. I wish to express appreciation to Professor Goldschmidt who suggested the problem and furnished invaluable help throughout its progress. Technical assistance by Dr. Werner Braun and services rendered by the personnel of Work Projects Administration, Official Project 465-03-3-192 are gratefully acknowledged.

## EXPERIMENTAL PROCEDURE

The mutants, including chromatin rearrangements, to be tested were introduced individually into a stock containing vg and three known dominigenes to determine whether they act as dominance modifiers for heterozygous vg. For the experiment a stock, called Doho to symbolize dominigene homozygous containing the three dominigenes and vg was used. This stock had been inbred with great care by Goldschmidt since he, simultaneously with Stanley, discovered the dominigenes in 1935. The stock was continued by single pair matings between flies with notched wings (kn). By this means flies expressing the dominance effect, heterozygous for vg in the presence of the dominigenes, were produced continuously. All matings made to test the dominance-modifying effect of introduced mutants, however, were crosses between vg Doho females and males from the stock being tested. The test cannot be used for sex-linked mutants. Thus the Doho first chromosome, containing the sex-linked dominigene, was present in all male offspring, along with the two autosomal dominigenes and vg, all in heterozygous condition, as it is known that this combination produces dominance of vg. It would be an advantage to test each mutant with the dominigenes in homozygous condition as well, but this proved technically impossible because the dominigenes occupy the first, second, and third chromosomes and do not have any visible expression when alone (for combination effects see Goldschmidt, 1937). The result of each test cross compared with the control crosses was interpreted to indicate the effect produced by the mutant stock introduced on the dominance of vg, under the controlled conditions of the experiment. A large-scale control experiment showed the complete recessiveness of vg when alone.

Ideally, isogenic stocks should be used so that unknown modifiers in the test stocks cannot influence the results. However, repetition of the test and control

crosses at different times, some of them one year apart, gave identical results. Furthermore, like results were obtained when the mutation was secured from different stocks or used in different combinations with other mutants. Thus the locus named below in each case was the one involved. Goldschmidt and Gardner (this volume) show that some mutants—black (b), for example, whose effect was already known to Dexter and Muller—enhance or inhibit identically the dominance of Beaded (Bd) and vg plus the dominigenes. This suggests that the same mutant loci may enhance or inhibit the dominance of different mutants concerned with scalloping. Such a fact becomes important when definite rules appear, e.g., that b inhibits not only vg dominance but also such genetically different types of scalloping as Bd.

A cross of a homozygous Doho vg female with a normal male ought to yield all sons kn but only a few daughters kn, and did so when the experiments were started. But soon afterward the stock reverted to a heterozygous balance for one of the dominigenes of the type analyzed by Goldschmidt (1937). Only half the sons from such a cross were kn, having all three dominigenes and vg/+. This condition of the stock, which then remained constant, was considered an advantage because it produced two classes of males, one with three and one with two dominigenes, therefore permitting a check in  $F_1$  upon two different combinations. Inasmuch as the controls Doho-vg  $\times$  Oregon remained constant, the genetic constitution of Doho was not further tested.<sup>1</sup>

Wild Oregon-R stocks, inbred for several years, were used for control crosses. They contained no dominance modifiers for vg or for Bd. Fifty per cent of the males resulting from vg Doho females  $\times$  Oregon-R males had notched wings: of 1076 males, 537 were kn and 539 were normal. Only about 1 per cent of the females were kn. The expectation from a cross of vg from this Doho stock by a mutant stock is the following: males, 50 per cent kn, 50 per cent normal; females, 1 per cent kn, 99 per cent normal. A significant deviation from this result was interpreted to indicate the influence of the introduced mutant upon the action of the dominigenes, provided that identical results were obtained with stocks of different origin containing the mutant.

When more than half of the males are kn, some of the potentially normal males are apparently enhanced to scalloping; when less than half are kn, the normal dominigene action is apparently inhibited. When more than 1 per cent of the females are kn, the dominigene action, ordinarily ineffective with the sex-linked dominigene in heterozygous condition, has been enhanced or pushed above the threshold of action. The percentage of kn females (minus about 1 per cent) measures the enhancing action of the females. The majority of mutants with positive effect did not act directly as dominance modifiers of heterozygous vg but only in the presence of the three standard dominigenes. Specific mention will be made of mutants which had a positive action in the absence of the dominigenes.

<sup>1</sup> The reliability of the following analysis is in no way weakened by the constitution of the Doho stock. This constitution, whatever it may be, is typical, and therefore any difference between controls and experiment is significant. Since this paper was written Mr. Richard Blanc has done much work trying to disentangle the genetics of this Doho stock. The results are not yet satisfactory and the work is being continued.

—R. B. GOLDSCHMIDT.

Crosses and appropriate controls were raised on the same food, with the same amount of moisture, and kept in the same carefully controlled constant temperature incubator, at 25° C. The temperature did not vary more than 1° C. during the entire experiment. The offspring resulting from each pair of flies were carefully classified according to the method used by Goldschmidt (1935, 1937). The classes range from I to X, representing wings ranging from slightly nicked to the vestigial wing with only the stump remaining. In the description of crosses the parent first indicated is always the female.

### EXPERIMENTAL DATA

Goldschmidt (1935) observed that one locus (b) had an inhibiting effect upon dominigene action in whatever combination it was introduced, and that an inversion (Cy) had an enhancing effect. He noted also that certain inversions have a strong enhancing effect upon the scalloping action of the Bd locus. Therefore my interest was centered upon the action of inversions, but in addition many mutant loci were tested, all according to the method outlined in the preceding paragraphs.

#### MUTANTS WITHOUT SIGNIFICANT EFFECT

Among 111 different stocks tested, the following mutants proved to have no significant effect on dominigene action: expanded (ex), fat (ft), dumpy (dp), reduced (rd), ancon (an), hook (hk), roughest (rh), Bristle (Bl), cinnabar (cn), bat (bat), curved (c), narrow (nw), purpleoid (pd), speck (sp), sepia (se), thread (th), radius incompletus (ri), peach (p<sup>p</sup>), Stubble (Sb), spineless (ss<sup>a</sup>), bithorax (bx), stripe (sr), crossveinless (cv), Prickly (Pr), claret (ca), bent (bt), cubitus interruptus (ci), eyeless (ey). A much larger proportion of mutants will probably fall in this group when a more extensive study is made.

#### MUTANTS ACTING AS INHIBITORS

Sixteen of the tested mutants inhibited the action of the dominigenes and normal wings tended to be produced. Table 1 summarizes the results of each series of crosses, gives the number of normal males (+ ♂) and kn males (kn ♂), the degree of kn action (♂ kn class), and the percentage of kn individuals below the 50 per cent which appear in the controls (♂ per cent inhibition). About 1 per cent of the females resulting from most of these crosses were kn, similar to the controls. In a few crosses a higher percentage was obtained. The results obtained among the females are listed under the headings, + ♀, kn ♀, ♀ kn class. All kn females in this group fall in Class I.

Some inhibitors have been identified with chromosome rearrangements, but most of them have not been classified. As is shown in table 1, the Plexate deficiency (Px<sup>2</sup>) is a pronounced inhibitor. The Blond translocation (T[1:2] Bld) also inhibits dominigene action markedly in the ♂. Other second chromosome inhibitors are the following: thickoid (tkd), black (b), jaunty (j), light (lt), chubby (ch), lanceolate<sup>2</sup>, (ll<sup>2</sup>), and blistered (bs<sup>2</sup>). We shall later see that bs is an enhancer. None of these have been definitely identified with chromosome rearrangements. In the third chromosome, hairy (h), Deformed (Dfd),

maroon (ma), curled (cu), glass (gl), Hairless (H and H<sup>2</sup>) inhibit kn action. Most of these have not been studied cytologically. Bridges (Morgan, Bridges, Schultz, 1937) reports that the salivaries are apparently normal at the Dfd and H loci.

#### THE ENHANCING ACTION OF INVERSIONS

Twenty-two stocks known to carry inversions, all of which had been analyzed cytologically, were secured for the study. Tables 2 and 3 summarize the results obtained from crosses between vg females from the Doho stock and males from stocks known to carry inversions in the second and third chromosomes. With

TABLE 1  
HETEROZYGOUS MUTANTS WHICH INHIBIT DOMINIGENE ACTION  
CHROMOSOME II

Mutant	Locus	+σ	kn σ	Per cent σ <sup>2</sup> Inhibited	knσ <sup>2</sup> class	+♀	kn ♀	kn ♀ class
tkd.....	30.	91	24	29	II	111	0	I
b.....	48.5	108	25	31	I	139	1	I
j.....	48.7	183	77	20	I	288	2	I
lt.....	55.	76	38	17	II	120	0	
ch.....	72.5	111	73	10	II	203	2	I
ll <sup>2</sup> .....	106.7	159	60	23	II	215	26	I
bs <sup>2</sup> .....	107.3	145	53	23	II	255	0	
Bld (T[1:2]) ..		112	32	28	I-II	91	23	I
Px <sup>2</sup> (Df 107-)		69	3	46	I-II	94	0	

#### CHROMOSOME III

h.....	26.5	225	28	39	I	282	0	
Dfd.....	47.5	115	35	27	I	142	0	
ma.....	49.7	54	23	17	I-II	77	2	I
cu.....	50.	69	43	12	I-II	123	1	I
gl.....	63.	175	30	35	I	202	8	I
H.....	69.5	347	2	49	I	331	0	
H <sup>2</sup> .....	69.5	52	1	48	I	65	0	

each inversion symbol is given its location in terms of Bridges' reference system (Bridges, 1935) as determined by salivary examination, and the authority for the salivary analysis. The numbers of individual cultures are omitted and only sufficiently large totals are given. Results obtained from individual crosses involving inversions of special interest are discussed separately. In all crosses except one (with Florida), only flies known to carry the inversion in question are listed. The other 50 per cent serve as a control if they do not carry another balancing inversion. The results of crosses with stocks carrying balancers are listed with the inversion they carry. The presence of the inversion was ascertained by the use of markers, either associated with the inversion or located in the opposite chromosome. The percentage enhancement of scalloping in males is calculated on the assumption that exactly 50 per cent of the males would be kn without such action. Enhancement for males

means, therefore, production of scalloping in the otherwise normal Doho group. Enhancement in the otherwise kn group would become visible by a higher grade of scalloping. Enhancement in females means scalloping above 1 per cent in spite of heterozygosity of the sex-linked dominigene.

The results (tables 2 and 3) show that of the 22 inversions successfully studied, 21 enhance the action of the dominigenes, some only in the males, others both in females and males. Usually, only the lower classes of kn action (kn I-III) were encountered; In(2LR)Gla, however, was found to enhance kn action markedly. Results of crosses involving other inversions which enhance kn action vary considerably, as the column indicating the per cent of enhancing shows. The size of the inversion and the effect it produces do not seem related. No general rule can now be made to relate the effect to a particular region in the chromosomes, although some parts of the chromosome, when rearranged, seem to cause a greater abnormality in the wings than in other regions. Table 2 shows that the enhancing effect of inversions is greater in the right than in the left arm of the second chromosome, and the combined action of right and left arm inversions is cumulative. In the third chromosome (table 3) the region between 84 and 96 apparently has a greater effect when broken than do other parts of the chromosome.

As all these inversions cannot be assumed to carry similar modifying genes, we are facing a dominance-shifting position effect of a type comparable to that of cubitus-interruptus in translocations involving the fourth chromosome (Dubinin, 1936). The inversions may act only through those dominigenes in the homologous chromosome, or more likely, in view of the action in females, they may act upon the whole system of dominance modification. Goldschmidt and Gardner (this volume) show that in Beaded, only inversions in the homologous chromosome exercise this position effect (see also Goldschmidt, Gardner, and Kodani, 1939).

A few of the inversions listed deserve special consideration. Five different stocks containing inversions usually associated with the Curly phenotype were included in the study. Two of these stocks contain Curly inversions in the left arm of the second chromosome (In[2L]Cy t differs from In[2L]Cy). One contains only the right-arm inversion. Two carry both left- and right-arm inversions. The inversions in the two stocks last mentioned are apparently identical except that one has been separated from the visible character. As shown in table 2, all five stocks enhance kn action. The percentage in excess of the controls of kn males produced by the left-arm inversion is 18 per cent, by the right-arm inversion, 44 per cent, alone, and by the two combined, 64 per cent. This suggests that the effect of the right and left arm is additive. The degree of kn action is similar for all three combinations (kn II-III).

When the present study was first begun, both Florida and Oregon-R wild stocks were used for control purposes. Soon some of the crosses involving Florida produced more kn males than were expected. A similar irregularity occurred in crosses between Florida and Bd (Goldschmidt and Gardner, this volume). In an attempt to explain the discrepancy, salivary chromosomes were examined and a large inversion was seen in the Florida stock (Goldschmidt,

TABLE 2  
INVERSIONS IN SECOND CHROMOSOME

Inversion	Location	Salivary analysis citation	+♀	+♂	kn ♀	kn ♂	kn ♂ class	Per cent enhanced	
								♀	♂
In(2L)NS.....	23F2-36F2	Morgan, Bridges, Schultz (1936)	29	20	51	70	III	63	56
In(2L)Cy t. Su-S dp <sup>4</sup> pr.....	22D8-34A9	Morgan, Bridges, Schultz (1937)	314	148	12	210	II-III	3	18
In(2L)Cy b pr.....	22D1-33F	Morgan, Bridges, Schultz (1936)	110	106	0	152	II-III		18
In(2R)Cy L <sup>1</sup> sp <sup>2</sup> .....	42A2-58A	Morgan, Bridges, Schultz (1936)	251	36	0	92	II-III		44
In(2L+2R)Cy.....	22D1-33F	Morgan, Bridges, Schultz (1936)	551	134	10	448	II-III	1	51
In(2L+2R)Cy, S <sup>2</sup> (not Cy phenotypically).....	42A2-58A	Morgan, Bridges, Schultz (1936)	133	25	7	115	II-III	4	64
In(2L)dp.....	42A2-58A	Bridges, D. I. S., 7:10.....	115	20	0	92	III		64
In(2R)Pn.....	27D1-48B1	Schultz and Bridges, D. I. S., 7:10	170	38	18	171	III	9	64
In(2L)Gla.....	40F-50E1	Morgan, Bridges, Schultz (1936)	4	5	337	228	VIII-IX	99	96
S Es (carried with inversions).....	27E-51D	D. I. S., 4:8.....	214	42	3	143	II-III		54
S sp (carried with inversions).....		D. I. S., 4:8.....	24	16	65	72	II-III	75	64

TABLE 3  
INVERSIONS IN THIRD CHROMOSOME

Inversion	Location	Salivary analysis citation	+♀	+♂	kn ♀	kn ♂	kn ♂ class	Per cent enhanced	
								♀	♂
In(3L)D.....	69D1-70D1-2	Bridges, D. I. S., 7:11.....	184	55	3	152	II	—	46
In(3L)D <sup>2</sup> .....	69D1-70D1-2	Bridges, D. I. S., 7:11.....	66	38	5	67	II	6	28
Florida (Probably In(3R)P <sup>a</sup> .....		Bridges, D. I. S., 7:12.....	401	218	148	342	II-III	26	22
In(3R)C1 3a.....	94E1-100F1	Bridges, D. I. S., 7:12.....	166	76	1	88	II-III		8
In(3R)C ca.....	94E1-100F1	Bridges, D. I. S., 7:12.....	290	101	8	186	II-III	2	30
In(3R)C.....	94F1-100F1	Bridges, D. I. S., 7:12.....	97	41	0	65	II-III		14
In(3R)hp.....	Unanalyzed	Bridges, D. I. S., 7:12.....	30	21	10	31	II	24	20
In(3R)Hu.....	84B-86C	Morgan, Bridges, Schultz (1937) ..	52	6	4	35	III	7	70
In(3L+3R)P.....	63C-72E	Bridges, D. I. S., 7:12.....	280	32	14	170	III	5	68
2C Dfd ca (carries Payne inversion).....	89C1-96A	D. I. S., 8:26.....	75	21	48	88	II-III	38	60
In(3LR)Cx, D.....	72A-95F	Morgan, Bridges, Schultz (1937) ..	154	119	5	61	I-II	2	32 <sup>b</sup>
In(3LR)Cx, D (repeat).....	72A-95F	Morgan, Bridges, Schultz (1937)	300	203	5	96	I-II	1	36 <sup>b</sup>

Gardner, and Kodani, 1939). The inversion is probably In(3R)P, which is reported to be widely dispersed in wild stocks (D. I. S., 7:12).

The Florida stock is not balanced and the chromosome carrying the inversion seems to segregate at random. Because it has no visible expression, inspection does not show which flies carry the inversion. If the increased kn action is caused by the inversion, as suspected, the results from crosses between vg Doho females and Florida males, selected at random, would be expected to be irregular. More than half the sons of each father carrying the inversion would be kn, whereas the sons of fathers not carrying the inversion would be expected to occur in the proportion of one kn male to one normal male. The results from single pair matings between vg Doho females and males

TABLE 4  
CHI-SQUARE TESTS OF DOHO VG ♀ × FLA ♂ ON BASIS OF 1:1 EXPECTANCY

Culture	+♀	+♂	kn ♀	kn ♂	Class	$\chi^2$
3522.....	48	19	8	41	II-III	8.06
3523.....	32	21	5	17	II-III	1.68
3524.....	27	7	2	21	II-III	7.00
3525.....	42	23	2	24	II-III	0.02
3526.....	17	9	3	16	II-III	1.96
3527.....	38	22	8	35	II-III	2.96

selected at random from the Florida stock bottles are compiled in table 4. The deviation from the expected ratio is significant in the results of 3522 and 3524, but not in 3523, 3525, 3526, and 3527. The inversion was assumedly present in the fathers used in the two crosses that did show the significant deviation, and absent in those that did not. In the parallel case with Bd this was proved cytologically.

Recent investigation by Bridges (Morgan, Bridges, Schultz, 1937) has shown that the dominant mutant D and its allele D<sup>s</sup> are associated with a short inversion in the left arm of the third chromosome. The present study shows that both enhance kn action.

Another D inversion, In(3LR)Cx, D, made at Austin, Texas, by X-raying D flies, was a pronounced inhibitor of kn action. Results of two series of crosses (table 3) show that 32 per cent and 36 per cent, respectively, less than the expected 50 per cent of kn males were produced when In(3LR)Cx, D was present. This inversion is similar to the other D inversions, but contains, in addition, breaks at 72A, 80D, 84E, 85E, and 95E (Morgan, Bridges, Schultz, 1937), and extends from the locus of D to somewhat beyond the locus of H. This inversion, unlike other D inversions, includes the locus of H, which is a strong inhibitor, suggesting that something in this region may counteract the effect of the dominigenes and enhance the production of normal wings.



# DOMINANCE-ENHANCING ACTION OF MUTANTS GENERALLY ASSUMED TO BE POINT MUTANTS

In addition to enhancers identified with inversions, there are several others which are supposed to be point mutations, though most of them have not been thoroughly studied cytologically. Some are thought to be associated with chromosome rearrangements. The results of crosses embracing these enhancers are tabulated in table 5, which gives the actual numbers of normal and kn

TABLE 5  
OTHER HETEROZYGOUS MUTANTS ACTING AS ENHANCERS

## CHROMOSOME II

Mutant	Locus	+♂	kn ♂	Per cent ♂ enhanced	kn ♂ class	+♀	kn ♀	Percent ♀ en- hanced
al.....	0.	114	174	20	II-III	308	0	
net.....	0.	4	104	92	II-III	101	22	17
pi.....	10.	19	89	64	II-III	123	0	
d.....	31.	25	108	62	II-III	112	7	5
d (repeat).....		30	165	70	II-III	215	4	1
pr.....	54.5	36	101	48	II-III	153	3	1
mi.....	104.5	21	103	66	II	143	2	
bw.....	104.5	23	95	60	II	107	16	13
bs.....	107.3	23	130	70	II	141	8	4
Wr.....		8	78	82	II-III	81	8	8

## CHROMOSOME III

h <sup>a</sup> .....	26.5	57	141	62	II	199	0	
Gl.....	43.4	0	24	100	III-IV	16	7	29
DI <sup>4</sup> .....	66.2	26	215	78	IV	158	89	35
DI <sup>5</sup> .....	66.2	1	23	92	IV	33	4	10
e.....	70.7	204	272	14	II-III	353	2	

males and kn classes, and the percentage of kn males in excess of those in the control. In most crosses, as in the controls only, about 1 per cent kn females were produced. Where more than 1 per cent kn females were produced the percentage is given under "Per cent ♀ enhanced." The kn females were present in the same classes as the males.

In addition to the enhancers listed in table 5 there are a few other mutants with a slight but significant enhancing effect. Four members of the Lobe (L) series of alleles were tested, and each slightly enhanced the action of the dominigenes. The similarity of the results produced by each of the four alleles indicates that certain embryological process may be influenced in much the same way by each member of the series.

The results parallel those found for inversions. It would be gratifying to find a rule concerning the phenotypic action of these loci as related to their dominance-enhancing action. It is remarkable how many of the strong en-

hancers have an effect upon wing venation, namely net, bs, Wr, D1<sup>4</sup>, D1<sup>5</sup>. There is a close, but as yet unexplored, developmental relation between scalloping and extra venation. In pure Bd stock the nick is sometimes replaced by an extra vein (Goldschmidt and Gardner, this volume). This may therefore be significant and must be kept in mind in future phenogenetic work.

#### INHIBITORS AND ENHANCERS COMBINED

Experiments combining both opposite actions are of interest as the results may indicate whether all the actions described can be considered to affect the same embryological processes. Though a final answer cannot be derived from such

TABLE 6  
INHIBITORS COMBINED WITH ENHANCERS

Mutant	+♂	kn ♂	Class
b cn.....	99	21	I
b cn.....	110	31	I
b.....	107	25	I
cn.....	145	205	I-II
b j pr.....	136	157	II-III
b j.....	64	17	I
b.....	107	25	I
j.....	183	77	I
pr.....	36	101	II-III
al b c sp.....	48	55	II
al.....	114	174	II-III
b.....	107	25	I
c.....	36	23	II
sp.....	73	74	II
h.....	225	28	I
h w <sup>a</sup> se.....	175	47	I
w <sup>a</sup> se.....	12	14	II

experiments, it may be argued that a simple quantitative relation between the actions of enhancers and inhibitors is in favor of an action upon the same process, whereas irregular results would point to different actions. When mutants whose effectiveness as enhancers or inhibitors is known are combined in the same stock, the extent to which the offspring will be scalloped can sometimes be predicted. Often two mutants, having about equal influence in opposite directions, offset each other and the offspring show ratios similar to those of the control crosses. For example, the mutant b inhibits kn action to about the same extent when alone as when combined in the same stock with such neutral mutants as cn, j, c, sp (table 6). When, however, b is combined with pr, which is known to enhance kn action with about the same intensity as b inhibits kn action, the ratio is approximately 1 kn ♂ to 1 + ♂. This indicates that the inhibiting effect of b is offset by the enhancing effect of pr. Similar results are obtained when h, a pronounced inhibitor, is combined with other mutants whose effect on action is known, as shown by table 6.

Since the inversions  $\text{In}(2\text{L}+2\text{R})\text{Cy}$ ,  $\text{In}(2\text{LR})\text{Pm}$ , and  $\text{In}(3\text{R})\text{Sb}$  are enhancers of dominigene action, and  $\text{H}$  is a strong inhibitor, crosses were made to determine the effect produced when different inversions are combined in the same stock with  $\text{H}$ , (table 7). Males from a stock containing all three inversions and  $\text{H}$  were crossed with  $\text{vg}$  Doho females. The results show that  $\text{H}$  still acts as an inhibitor. Without  $\text{H}$  all the inversions enhance  $\text{kn}$  action. Genetic

TABLE 7  
 $\text{vg Doho } \varnothing \times \text{In}(2\text{LR})\text{Pm}/\text{In}(2\text{L} + \text{R})\text{Cy}, \text{In}(3\text{R}), \text{Sb} / \text{H} \sigma^7$

Culture	Class	+♀	+♂	kn ♀	kn ♂	kn class
3098.....	<i>Cy Sb</i>	7	2	0	6	II-III
	<i>Cy H</i>	3	7	0	0	
	<i>Pm Sb</i>	11	1	0	10	II-III
	<i>Pm H</i>	6	12	0	0	
3099 .....	<i>Cy Sb</i>	15	3	0	4	II-III
	<i>Cy H</i>	12	8	0	0	
	<i>Pm Sb</i>	9	0	1	10	III
	<i>Pm H</i>	14	13	0	0	
3100....	<i>Cy Sb</i>	2	1	0	6	II-III
	<i>Cy H</i>	4	5	0	0	
	<i>Pm Sb</i>	0	1	0	5	III
	<i>Pm H</i>	5	4	0	1	I
3228.....	<i>Cy Sb</i>	0	0	0	4	II
	<i>Cy H</i>	3	3	0	0	
	<i>Pm Sb</i>	4	1	0	4	III
	<i>Pm H</i>	3	2	0	0	
Total .....	<i>Cy Sb</i>	24	6	0	20	II-III
	<i>Cy H</i>	22	23	0	0	
	<i>Pm Sb</i>	24	3	1	29	II-III
	<i>Pm H</i>	28	31	0	1	I

experiments did not reveal more. But phenogenetic experiments carried out at this laboratory both by Braun (1939) and myself tend to show that the action is upon relative rate of differentiation.

#### STRONG DOMINANCE ENHANCERS ACTING IN THE ABSENCE OF STANDARD DOMINIGENES

For all combinations reported thus far the dominance-enhancing action of loci and inversions required the presence of the sex-linked dominigene  $\text{ct}^{\text{do-vg}}$  and at least one of the two autosomal dominigenes  $\text{A}$  and  $\text{B}$  in order to be effective upon the  $\text{vg}$  heterozygote. One exception was already mentioned, the Glazed Inversion,  $\text{In}(2\text{LR})\text{Gla}$ . This inversion added to heterozygous  $\text{vg}$  alone, that is, the combination  $\text{vg}/\text{In}(2\text{LR})\text{Gla}$  without dominigenes, shows scalloping

(fig. 1b). Following are the results of the cross (3649-53) between vg females without dominigenes and males carrying the Gla inversion: 77 + ♀♀, 26 + ♂♂, 78 kn ♀♀, 88 kn ♂♂, kn classes I-VII; 50 per cent of the females and 77 per cent of the males were kn. When the dominigenes were present in addition, practically all the offspring were kn, classes VIII-IX (table 2). Furthermore, a lower viability was noted among males containing both Gla and ct<sup>do-vg</sup>. Gla has the effect upon the cornea of the eyes which is expressed in the name glazed. But this inversion alone has no wing effect whatever. This must be emphasized in view of the data on facet.

Another mutant with effects resembling that of the Gla inversion is contained in a stock carried in this laboratory under the name of Doma (fig. 1c). It is a sex-linked powerful dominance enhancer for vg, different from ct<sup>do-vg</sup>, with no visible expression when alone, homozygous viable, and effective, like

TABLE 8  
CROSSES OF DOHO WITH DOMA

Culture	Cross	vg ♀	vg ♂	kn ♀	kn ♂	+ ♀	+ ♂
2609,9a	Doho (kn III) × Doma (kn VII)	35	29	34	55	52	31
2610,11	Doma (kn VII) × Doho (kn III)	27	25	23	43	40	20
2614,15	Doma (kn III) × Doho (kn III)	20	29	28	54	56	36

Gla, in the absence of the standard dominigenes. It could not be localized with certainty, but there is a strong indication that it is an allele of facet, and it is therefore called fa<sup>do-vg</sup>. It was originally isolated by Goldschmidt from a plexus (px) stock, was subsequently lost, and was found there again in 1935 by Mr. Ma, working in Goldschmidt's laboratory. It is carried in the Doma stock in the same way as the Doho enhancers: a stock homozygous in fa<sup>do-vg</sup> and containing vg is continued by breeding from scalloped individuals vg/+ and segregates in 1 vg: 2 intermediate (kn): 1+. The flies of this combination with vg/+ are scalloped in the kn classes V-VIII, as opposed to I-III in the Doho stock. An actual count of such a segregating bottle is 128 vg: 390 kn V-VIII: 184+. The enhancer fa<sup>do-vg</sup> is also recessive, as heterozygous females are not scalloped. The effect of this strong enhancer can be still increased to an average of kn class VIII if further enhancers are present.

Professor Goldschmidt carries another stock, symbolized F<sub>20</sub>sn Ma I kn VIII, which produces more extreme kn action and a wing pattern slightly different from that of the original Doma stock (fig. 1d). This stock has been obtained by selection from the Doma stock. A number of experiments have been carried out for the purpose of determining where the extreme modifying action is localized. The results strongly suggest that the enhancing action may be modified by a factor located in the extreme left end of the second chromosome.

One should expect that a combination of fa<sup>do-vg</sup> and ct<sup>do-vg</sup> in the same individual would be intermediate if they are alleles. If not, either the females containing both ought to be normal, or the slight heterozygous effect char-

acterizing both could be additive. Therefore Doho and Doma, both heterozygous for *vg*, were crossed in both directions (table 8). The fact that all *kn* offspring actually fell into the lower *kn* classes I-III, and that in the females the genetic constitution of the X chromosomes was  $ct^{do-vg}/fa^{do-vg}$ , points to non-allelism and suggests additive heterozygous action. The numbers of males from the reciprocal crosses was similar, suggesting that the A and B dominigenes act as inhibitors upon  $fa^{do-vg}$ , a strange result. Thus, a segregation of 1 *vg*: 2 *kn*: 1 + is expected and obtained for the males. The females should be normal (except for about 1 per cent *kn*) if the two enhancers are not alleles, but all *kn* if they are. Actually about half the females are *kn*, which shows that in the double heterozygote  $fa^{do-vg}/ct^{do-vg}$  the small heterozygous effects are additive and increase the *kn* females to about 50 per cent.

The action of  $fa^{do-vg}$  can, however, be greatly enhanced by a combination with the inversion *Gla*. Results obtained when *Gla* was crossed into the Doma stock were similar to those involving the Doho stock, but the degree of notched action was much greater (*kn* VIII-IX). Following are the actual results of crosses (2781-5):

$$vg \text{ Doma} \times In(2LR)Gla = 0 + \text{♀ ♀}, 0 + \text{♂ ♂}, 62 \text{ } kn \text{ ♀ ♀}, \\ 58 \text{ } kn \text{ ♂ ♂}, \text{ } kn \text{ class IX.}$$

The wings were almost completely absent and approached *vg* in appearance (figs. 1e, f); many would be classified as *vg*<sup>strap</sup> if compared with members of the *vg* series. A reciprocal cross (2699) was also made with results as follows:

$$In(2LR)Gla \times vg \text{ Doma} = 0 + \text{♀ ♀}, 6 + \text{♂ ♂}, 28 \text{ } kn \text{ ♀ ♀}, \\ 29 \text{ } kn \text{ ♂ ♂}, \text{ } kn \text{ classes VII-IX.}$$

Two differences which may be significant resulted from these reciprocal crosses. In the first place, the degree of *kn* action was slightly lower, especially in the male offspring, when *Gla* mothers were used. Secondly, a few normal males were produced when the mothers carried the inversion. Sons from mothers containing the inversion would get normal sex chromosomes which would not contain the sex-linked dominigene. The autosomes would be similar in the results of both crosses. The lower degree of wing destruction and the few normal males produced from *Gla* mothers indicates that the greater effect in the reciprocal cross, both in numbers of *kn* individuals and in degree of *kn* action, is produced when both the inversion and the sex-linked dominigene are present.

#### ADDITIVE ACTION OF LOCI PRODUCING SCALLOPING AND THEIR INTERACTION WITH THE DOMINANCE MODIFIERS

According to standard genetic conceptions the presence of two different recessive mutants in heterozygous condition in the same individual will not have any visible effect, even if both mutants affect the same character. A dynamic conception of dominance, however (Goldschmidt, 1938), permits an additive action of two completely recessive mutants, both present in heterozygous condition, if both affect the same embryological reaction. Such a reaction might surpass the threshold of visible action when both simplex effects are combined. The actual occurrence of such an effect would be of great importance

for a theory of dominance as well as a theory of action of the mutant loci. When a dominant and a recessive mutant in heterozygous condition are combined, the situation is similar; according to standard conceptions only the dominant effect ought to become visible.

Facet is a sex-linked recessive mutant affecting the eye structure, which simultaneously has a small effect upon the wings, as a small percentage of the individuals homozygous for *fa* are nicked. Notches are dominant deficiencies including the *fa* locus producing considerably notched wings, presumably by simplex action of the hypomorphic  $+^{fa}$  locus. This assumption will not be questioned here. Xasta is a translocation ( $T[2:3]Xa$ ) which causes scalloping of a definite type (fig. 1*g*). Combining these three "mutants" with heterozygous vestigial alone, and with vestigial plus the dominigenes, the following results were obtained: Perhaps the most extreme change in wing pattern was observed when the translocation  $T(2:3)Xa$  was crossed into the Doho stock. When combined with *vg* and the dominigenes (Doho) a different pattern is produced (fig. 1*h*). The pattern obtained when *vg* is present, without the dominigenes, is much the same as that produced by *vg* and the dominigenes (fig. 1*i*). The dominigenes, without *vg*, produce a less striking but characteristic change in the wing pattern (fig. 1*j*). These facts suggest that the major effect is produced by *vg* and not the dominigenes; that is, the additive effect of  $vg/+$  and  $Xa/+$ . The wings are longer and more like the normal wild type when *H* is present in the chromosome opposite the translocation than when the Oregon-R chromosome is opposite  $T(2:3)Xa$ . *H* strongly inhibits the action of the dominigenes as reported above, and enhances the production of normal wings.

Similar results were obtained for the locus *fa*. Repeated tests show locus *fa* to be completely recessive. But females heterozygous for both *vg* and *fa* are about 33 per cent *kn*, thus showing again the additive action of two recessives in simplex condition. This additive effect is, however, not very strong, though stronger than the homozygous *fa* effect, which produces about 10 per cent notched flies. The same action appears if  $vg/+$  is combined with heterozygous Notch, as the dominant effect of Notch is not greatly enhanced, so far as indicated by the degree of notching. Results (3645-48) show that all females (males are lethal) are *kn* III-VI, which is not much higher than the degree of *kn* action shown by flies from the Notch stock.

These results suggested a test of the influence of the dominigenes of the Doho stock upon the double heterozygote  $vg/+$   $fa/+$ . Table 9 shows the results. A comparison with the controls shows an extreme cumulative action of the heterozygous recessives  $vg/+$ ,  $fa/+$ , and  $ct^{do-vg}/+$  in the presence of *A* and *B*. Practically all of these females are *kn* and of a higher degree than otherwise (fig. 1*k*). This result, compared with the other combinations of the same loci, is highly suggestive, in the sense that two heterozygous actions might add up to the threshold of visible action. In all these experiments the eye effect of facet remains completely recessive. Interestingly, the Doho dominigenes without  $vg/+$  do not enhance heterozygous *fa* to the point of a visible *kn* effect. If, however, *fa* males carry in addition the dominigenes *A* and *B* without *vg* they are all *kn*, whereas only about 10 per cent are *kn* for *fa* alone, thus showing

that these dominigenes act also upon the *fa* effect. Males with *fa*, *A*, *B*, and *vg*/+ (without *ct<sup>do-vg</sup>*) are one class more *kn*.

To return to the strong sex-linked dominigene *fa<sup>do-vg</sup>* of the Doma stock: this dominigene seems to be an allele of *fa* without visible effect, just as *ct<sup>do-vg</sup>* is to *ct*. The reason can be given now that effects of *fa* in combination with *vg* have been analyzed. The *vg* Doma females, *vg/vg fa<sup>do-vg</sup>/fa<sup>do-vg</sup>* were crossed to *fa* males. All heterozygous daughters were *kn*. In addition they showed the eye character facet, which otherwise is completely recessive; *fa<sup>do-vg</sup>* must therefore be an allele of *fa* or a deficiency including the *fa* locus, which alone does not produce a wing effect. A deficiency is not likely, because *fa<sup>do-vg</sup>* can be

TABLE 9  
CROSSES OF *vg* DOHO WITH FACET

Culture	Cross	+ ♀	+ ♂	kn ♀	kn ♂	Class
2736	<i>vg</i> Doho × <i>fa</i> . . . . .	4	3	35	53	II-IV
2737	<i>vg</i> Doho × <i>fa</i> . . . . .	0	0	61	43	II-IV
Total		4	3	96	96	
2511,12	<i>vg</i> Doho × Ore (control) . .	103	48	0	51	II

combined with the notch deficiency without lethality. Mr. Kodani was kind enough to make a salivary analysis and found no deficiency. Such flies look like *vg<sup>trap</sup>*, thus showing an extreme enhancement upon *vg*/+. Oliver, (1937) suspects that *fa*, in itself, is a deficiency.

#### SOME PRELIMINARY DATA ON THE PHENOGENETIC BASIS OF DOMINIGENE ACTION

Dominance has taken on a somewhat different aspect now that Goldschmidt (1927) and Wright (1934) have shown that it is concerned with the physiology of development and not with the mechanism of inheritance. The results of the present study show that heterozygous flies are on one or the other side of a threshold, the limits of which are established by the strange quantitative relationships of the actions of all the loci, inversions, translocations, and deficiencies in homozygous and heterozygous condition which have been analyzed. Moreover, all the different actions affect but one developmental reaction. A further attack upon the problem must therefore be phenogenetic.

Results of studies of Gonzales (1923), Csik (1935), and Brierley (1938) show that the mutant *vg* has a decidedly detrimental effect on the duration of life of the flies; they also show that the mutants, plexus (*px*), Lobe (*L*), arc (*a*), cut<sup>6</sup> (*ct<sup>6</sup>*) and others affect the viability of flies which carry them. Inversions carried in laboratory stocks lower it. The present study has shown that at least some of the dominigenes also lower it. Flies carrying dominigenes and *vg* require a longer developmental period than *vg* flies. These facts demonstrate that there is a relationship between dominigene action and the general rate of

development and viability of the flies (see also Goldschmidt and Gardner, this volume).

Green and Oliver (1940), dealing in part with the same problems, have recently introduced three genetic factors, two Minutes and a duplication, which prolong developmental time into the *vg* heterozygote. Scalloping was increased, but not in direct proportion to the retarding of development.

### SUMMARY

(1) Twenty-eight mutant stocks had no effect upon the action of the standard dominigenes modifying the dominance of vestigial; 16 mutant stocks, including at least 2 chromatin rearrangements, inhibited the action of the dominigenes; 14 enhanced the action; 21 inversions enhanced and 1 inhibited dominigene action.

(2) Evidence gained from combining inhibitors and enhancers indicates that they affect the same embryological processes.

(3) Some dominance enhancers, such as *In*(2LR)*Gla*, *fa*<sup>do-vg</sup>, *T*(2:3)*Xa*, and *fa*, act in the absence of the standard dominigenes.

(4) The action of different loci which produce scalloping is additive when the loci are combined with dominance modifiers.



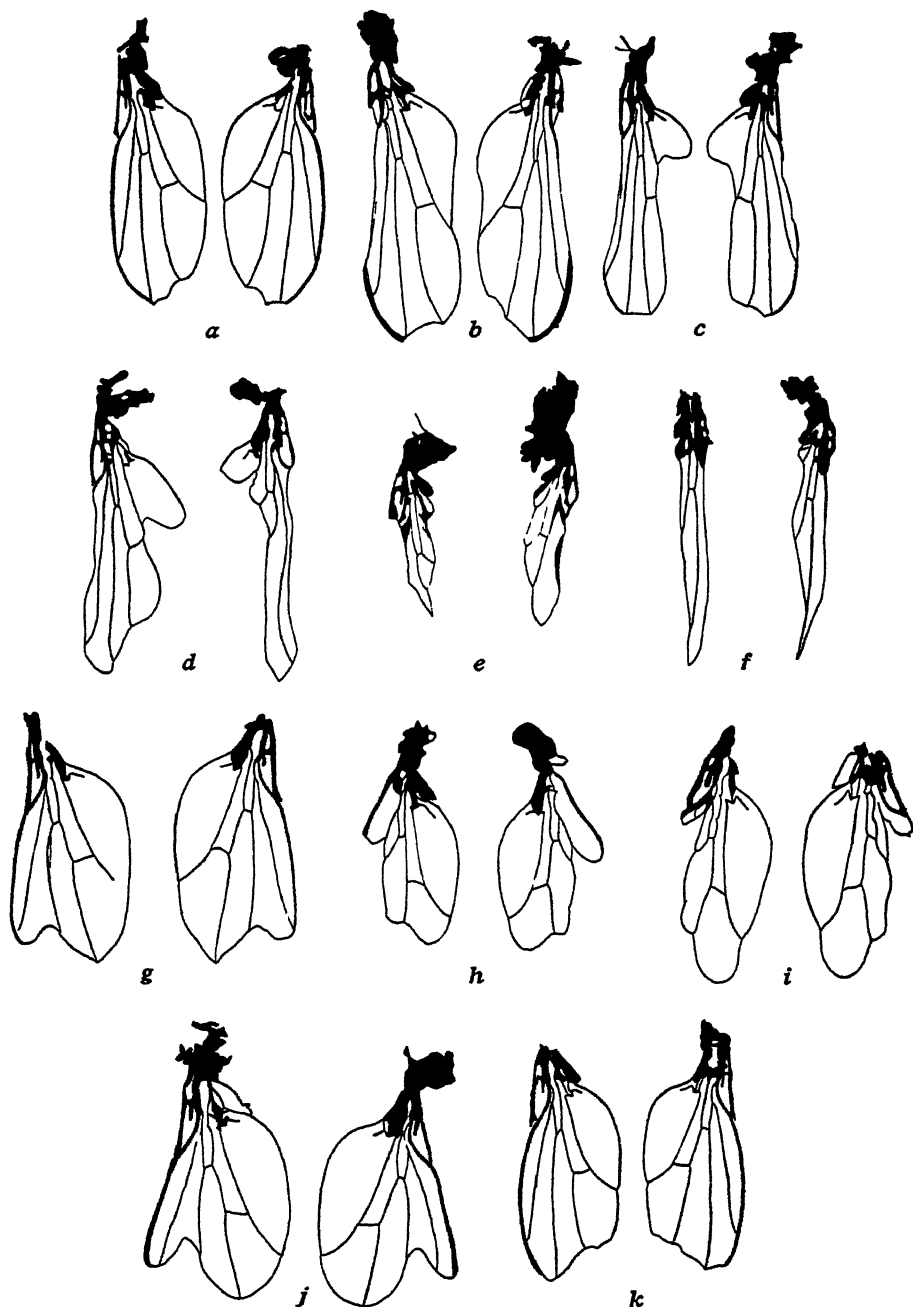


Fig. 1. Wings of: (a) ♀ heterozygous for *vg* and homozygous for the dominigenes *ct<sup>do-vg</sup>*, A, and B; (b) ♀ heterozygous for *vg* and *In(2LR)Gla*; (c) ♀ homozygous for *fa<sup>do-vg</sup>* and heterozygous for *vg*; (d) ♀ from selected *F<sub>25n</sub> Ma I kn VIII* stock; (e) ♀ heterozygous for *fa<sup>do-vg</sup>*, *vg*, and *In(2LR)Gla*; (f) ♂ heterozygous for *fa<sup>do-vg</sup>*, *vg*, and *In(2LR)Gla*; (g) ♀ carrying *T(2:3)Xa* (Oregon-R chromosome opposite translocation); (h) ♂ carrying *T(2:3)Xa*, *vg*, and Doho dominigenes, all heterozygous, from cross, *vg* Doho × *T(2:3)Xa*; (i) ♀ carrying *T(2:3)Xa* and *vg* (without dominigenes); (j) ♀ carrying *T(2:3)Xa* and Doho dominigenes (without *vg*); (k) ♀ heterozygous for *vg*, *fa*, *ct<sup>do-vg</sup>*, A, B.

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A FURTHER CONTRIBUTION TO THE ANALYSIS OF  
SCALLOPED WINGS IN DROSOPHILA  
MELANOGASTER

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# A FURTHER CONTRIBUTION TO THE ANALYSIS OF SCALLOPED WINGS IN DROSOPHILA MELANOGASTER

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IN *DROSOPHILA MELANOGASTER* Beaded (Bd) is generally considered a dominant mutation, expressing itself in the scalloping of the wings. Full expression of the character, however, requires the presence of certain enhancers in addition to the mutant itself. Much variation is observed in the wings of flies containing Bd and its modifiers in different combinations. One enhancer is known to be associated with an inversion, In(3R)C, and is effective when present in the chromosome opposite Bd (Muller, 1918). The senior author, many years ago (unpublished), noticed that other third chromosome inversions enhance Bd expression in the same way as In(3R)C (see also Goldschmidt, Gardner, Kodani, 1939). This observation is important because it suggests that the inversion itself produces the enhancing effect, rather than a mutant locus contained in the inversion. The enhancing action may therefore be regarded as a position effect. A similar type of action was found to influence the dominance of vestigial (Gardner, this volume).

The following account contributes further toward the analysis of scalloped wings in *Drosophila melanogaster*. All of our work was done at 25° C. with all conditions as identical as possible. Services rendered by the personnel of the Work Projects Administration, Official Project 465-03-3-192 are gratefully acknowledged.

## EARLY WORK WITH Bd

The mutant Bd was discovered by Morgan in May, 1910, from a culture of fruit flies which had been treated with radium (Morgan, 1911). One male fly was found originally, whose wings showed a few shallow marginal scallops which caused removal of the marginal bristles, parts of the marginal vein, and a slight amount of the blade of the wing. The intermittent or beaded appearance of the remaining parts of the marginal vein led to the name "Beaded" for the character. This one male was mated with a sister fly, from the same bottle, and the offspring with scalloped wings were repeatedly mated *inter se*. At first the Bd flies did not breed true but for many generations produced a number of normal winged offspring. Suddenly, after two years of selection, the stock changed to one which gave nearly all Bd offspring. The inversion opposite the Bd locus must have appeared at this time.

Morgan (1911) originally described Bd as a mutation, and it was commonly considered as such, but in some respects it seemed to defy Mendelian analysis. Dexter (1914) was the first to study the problem more thoroughly. He found that a Bd fly from the all-Bd stock, when mated to a normal fly "from a normal wild stock," produced a considerable number of flies with Bd wings in the first generation. It is unfortunate that we do not know more about Dexter's "normal" wild stock because recent studies have shown that some stocks, which have long been considered as normal wild stocks (e.g., Florida), carry factors which modify scalloping of the wing. At any rate, Dexter's observations indicated that the mutant is dominant. He observed, further, that the percentage of Bd offspring from crosses between "pure" Bd flies and "normal" flies was not constant, the number of Bd varying

from zero to 50 per cent. His results varied greatly, but showed that the average percentages of Bd winged offspring per mating fall into two groups, one at 10–15 per cent and the other at 30–35 per cent, making a distinct bimodal curve. About 60 matings were made by Dexter and only 2 of these gave 50 per cent Bd. Similar tests were carried out by other students (Bridges and Morgan, 1923) and the results showed repeatedly that Bd, when outcrossed to some "normal" lines, produced 25 per cent, or more Bd, and when outcrossed to others produced only about 5 per cent. We shall learn the explanation for some of these discrepancies.

Dexter carried his crosses to the  $F_2$ ,  $F_3$ , and  $F_4$  and made backcrosses, but the results were irregular and gave little satisfaction to strict students of Mendelism. Dexter noted that an  $F_1$  Bd fly, or even a fly of a later generation, which showed the Bd character, would sometimes produce as many Bd offspring when mated to wild, as a fly from a "pure" Bd stock when mated to wild. Some crosses suggested that a lethal factor came into play, others that a factor in the second chromosome intensified the dominance of Bd. Dexter thought this second chromosome modifier to be homozygous lethal.

Dexter next crossed other mutants into the Bd stock and found that some influenced the expression and some did not. The results of these crosses showed an even greater variation than those previously mentioned. The number of offspring was small, but indicated that the mutants pink, sepia, eosin, and perhaps vermilion and white have no enhancing or inhibiting effect. The mutants vestigial, antlered, and strap, which have now been shown to be vestigial alleles, he found to have a pronounced enhancing effect, and black inhibited the expression of Bd markedly. Black has now been shown to have a similar effect upon vg, (Gardner, this volume). As a possible explanation for the variability of Bd expression, Dexter suggests that "the dominance of the gene for beadedness varies in accordance with many other circumstances, among which are differences in other genes present." This conclusion in general is still correct.

Muller continued the study of Bd and completed the analysis of the genetic situation in a remarkable paper (Muller, 1918). From the results of a series of well-planned crosses, Muller concluded that several factors are involved and that the expression of Bd is a combination effect. Muller located the factor Bd in the third chromosome and concluded, as did Dexter, that the factor is homozygous lethal. This being true, all flies showing the character must be heterozygous for Bd. Therefore, Muller concluded, in the stock producing all Bd offspring, Bd must be completely dominant.

In addition to the Bd factor, Muller found another lethal factor (l) in the third chromosome. Unlike Bd, l produces no visible mutant effect. This lethal factor had apparently arisen by mutation in the selection of the original stock. In addition to these two factors, Muller found that the true breeding flies contained a factor which almost completely prevented crossing over in the part of the third chromosome where Bd and l are located. This crossover suppressor has since been identified as the inversion  $In(3R)C$  (Bridges and Morgan, 1923). This situation, involving two lethal factors, Bd and l, opposite each other, which effectively enforce the heterozygosity of each other, was called a case of "balanced lethals" (Muller, 1918). Muller believed that l could be separated by crossing over from the crossover inhibitor, the inversion.

Also in the third chromosome of the "pure" Bd stock, Muller located a factor which enhanced the Bd effect. This he called an intensifier. In the true breeding stock it was always in the third chromosome, opposite Bd. When Bd was outcrossed to stocks containing the mutants ebony and spread, the results indicated that these stocks also contained the intensifier. Furthermore, all stocks containing the intensifier (Bd, ebony, spread) contained  $In(3R)C$  which suppressed crossing-over in the region between pink (48.0) and rough (91.1) (Bridges and Morgan, 1923). The Bd, ebony, and spread stocks had all been derived from the same parental stock, namely, truncate, and therefore the crossover suppressors (the inversions) encountered were probably the same and were derived from the same source. The identity of these inversions has since been confirmed (Bridges and Morgan, 1923). Muller at that time assumed that the Bd intensifier and the crossover suppressor were different factors.

One other intensifier, described by Dexter, in the second chromosome, was also accepted by Muller. Muller found this factor to be homozygous viable, not homozygous lethal as Dexter had supposed.

### THE PHENOTYPE OF *Bd*, ITS EXPRESSIVITY AND PENETRANCE

We worked with Muller's original *Bd* stock, obtained 10 years ago from Russia, which still contained spineless in one third chromosome which Muller had crossed into the stock. Strangely enough, Deformed, also used by Muller in his analysis, occasionally appeared after outcrossing during the first year of the work (1934), though it is a dominant and should have shown up in the stock. For some reason dominance was suppressed. No further analysis was made. The phenotype of the stock was described originally by Morgan. Many counts were made in order to test the different types for genetical background. In the earlier work of the senior author five or six classes were distinguished:

- I. Normal.
- II. Normal but an extra vein in the fifth cell (not always checked).
- III. Nicked (*kn*) at tip of wing as in the *vg* compounds.
- IV. One wing scalloped at posterior margin.
- V. Both wings the same.
- VI. The same and also anterior margin beaded or scalloped.

Many selections were made for the different types over many generations without any effect. Occasionally, in a stock bottle which always had contained nearly all *Bd* flies, the number of normals increased and the number of the higher classes decreased. In pair cultures from these bottles and also in mass cultures most bottles returned to the old condition and it was impossible to select a low stock. Obviously, external conditions, already known to Dexter, influenced the expressivity and penetrance of *Bd* more than genetic variation present in the stock. Some of the recent experimental work with scalloping (see Braun, this volume) suggests that the effect of food upon speed of differentiation is involved, but no experiments were performed with *Bd* paralleling those with *vg*.

Morgan thought the presence of the extra vein instead of scalloping was a sign of an otherwise invisible *Bd/+* condition (see page 108), but there are reasons to assume a more complicated relation. In other experiments Goldschmidt (unpublished) noticed another extra vein type which required heterozygous vestigial for its expression. A usually homozygous lethal, dominant condition, located seven units from *vg* was involved, acting only in the presence of *vg*. Rare homozygous survivors were Beaded (phenotype). Without going into the details of the data, we mention these facts in order to show the developmental relations between loci producing scalloping and the production of an extra vein in the fifth cell.

Returning again to the *Bd* case, table 1 contains a few counts from pair matings and from the stock to show the occurrence of the different classes in the stock in 1935.



The table shows 31 individuals without scalloping (including extra vein) among 431; this is an expressivity of 92.8 per cent for scalloping if extra vein is not recorded. The mean class for females is 4.7; for males, 4.6. These and other data derived from extended observation of the stock show a negligible amount of genetic variation with respect to the expressivity of *Bd* in the balanced-lethal stock, this being about 92 per cent. Extreme effects of environment, however, were observed. After the work of Child (1939) we suspect that an action of Nipagin prolonging development was involved. This chemical compound was used while the great environmental influence was observed (see also Braun, this volume).

TABLE 1  
Bd PAIR MATING

Parents	Offspring classes											
	I		II		III		IV		V		VI	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1. Class VI.....	2	2	6	5	2	2	22	31	15	17	21	20
2. Class VI ( $F_1$ of I) ..	..	..	1	..	..	1	1	3	12	8	13	3
3. Class IV ( $F_1$ of I).....	..	1	5	..	2	..	13	15	18	18	2	1
4. Class VI ( $F_1$ of I).....	..	..	2	3	7	7	5	5	2	..	..	..
5. Class V ( $F_1$ of I).....	..	..	1	..	..	1	2	2	28	15	..	1
Stock.....	..	..	2	1	..	1	1	4	22	29	20	10
Total.....	2	3	17	9	11	12	44	60	97	87	56	35

#### PENETRANCE AND EXPRESSIVITY OF THE *Bd* LOCUS WITH AND WITHOUT THE INTENSIFIERS

The action of the dominant locus *Bd* in producing scalloping becomes visible when we remove the balancing inversion supposed to contain an intensifier, as well as the intensifiers found by Dexter and Muller in the all-Beaded stock.  $F_1$  of any cross removes the inversion from association with *Bd* and leaves other modifiers heterozygous. By backcrossing with marked second chromosomes the second chromosome of the original *Bd* stock can be replaced. By further crossing of phenotypically *Bd* individuals to a wild stock the eventual influence of the markers may be eliminated. By breeding  $F_2$  from a cross with a marked second chromosome (*Cy*), the combination of *Bd* without inversion but with its original second chromosomes may be obtained in  $F_3$ . Reciprocal crosses identify eventual first chromosome action in the males. The fourth chromosome was not checked. Table 2 summarizes the results without recording the individual crosses. In the genetic formulae of the  $F_1$ , II signifies the second chromosome of the *Bd* stock; *Inv.*, the inverted third chromosome from this stock; and *n*, the marked or unmarked second and third chromosomes from the other stocks. In table 2 the same classification is used as in table 1, except that classes V and VI were lumped and the extra vein (II) was not always checked.

TABLE 2  
F<sub>1</sub> Bd × SOMETHING; ALL OFFSPRING ½ II/N Bd/N, ½ II/N Inv/N  
-Bd FROM SAME INBRED BROOD

Cross	Classes of offspring										Penetrance (per cent)		Expressivity (class of scallop*)	
	I		II		III		IV		V/VI					
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂		
1. sy × Bd.....	466	412	not checked		8	10	39	36	28	22	27.6	28.4	4.03	4.2
2. Bd × sy.....	372	361	not checked		11	12	26	36	17	16	25.4	30.2	4.1	4.2
3. Bd × ss.....	164	196	85	3	5	2	6	3	3	1	11.2	5.8	3.9	3.9
4. ss × Bd 1.....	227	234	not checked		6	7	5	2	..	..	9.4	7.4	3.4	3.2
5. ss × Bd 2.....	212	342	189	10	6	9	5	3	..	1	5.5	7.4	3.5	3.4
6. S/Cy D/l × Bd.....	40	41	not checked			1					...	4.8	...	3
7. Bd × S/Cy D/l.....	171	138	not checked		4	1					4.6	1.4	3	3
8. S/Cy × Bd.....	223	233	not checked		10	20					8.6	15.9	3	3

<sup>a</sup> The presence of an extra vein (class II) was not considered as a kind of scalloping.

The penetrance is of course twice the actual percentage of scalloped flies, assuming an ideal 1:1 segregation for Bd and Inv. Figures in table 3 are comparable to those of table 2, although taken from experiments performed five years later with the same Bd stock after fresh inbreeding. The classification used here is a little different. Class III also contains minus individuals of the former class IV.

The tables show clearly:

(1) The penetrance of scalloping is typical for a given cross. As this is true for all individual crosses which have been summarized, it may safely be assumed that the Bd stock is not responsible for the difference (external conditions were, so far as possible, constant in all experiments). All crosses with small-eye (sy) had a high penetrance and therefore must have contained plus modifiers. These cannot be identical with the sex-linked mutant sy, as the re-

TABLE 3  
CROSSES COMPARABLE TO THOSE IN TABLE 2 (See text)

. Cross	I/II		III		Per cent penetrance		Expressivity	
	♀	♂	♀	♂	♀	♂	♀	♂
1. Bd × Oregon	784	746	1	5	.22	1.3	3	3
2. Oregon × Bd	520	493	9	..	3.4	...	3	..

ciprocal crosses show. The crosses with spineless (ss) and with the balanced stock show a much lower penetrance. The lowest penetrance was found in Oregon crosses. In addition to the crosses in table 3, numerous others were made as controls for further experiments. The penetrance kept always at the level of 2-5 per cent, mostly at  $\pm 2$  per cent.

(2) The first chromosome of Bd stock seems, from the crosses in table 2, to contain no modifiers for scalloping. Tables 3 and 4, however, based on crosses made years later, seem to indicate in the Bd stock a sex-linked condition which enhances scalloping, because the penetrance is consistently higher in males containing the Bd X chromosome, whatever the other chromosomes are.

(3) Where a check for extra vein was made, only few males showed it. The number of not scalloped females with extra vein suggests that extra vein is a heterozygous expression of Bd, as previously assumed by Bridges and Morgan. In the two sets in question the ratio of + females, to scalloped + EV females was 376 to 299. Nevertheless the foregoing conclusion is incorrect. Though most of the tested flies with extra vein turned out to be heterozygous for Bd, one F<sub>2</sub>, from F<sub>1</sub> parents with extra vein, consisted of 74 ♀♀ +, 55 ♀♀ EV, 90 ♂♂ +, 12 ♂♂ EV, and no Bd whatsoever, although in all F<sub>2</sub> containing Bd a rather high percentage of Bd was found (crosses with sy). Actually, a line breeding true for extra vein was established, but has not yet been analysed.

(4) Generally, penetrance and expressivity are correlated; with higher incidence of scalloping the higher grades appear. At the level of low penetrance only nicked wings appear, with an occasional fly with the nick at the posterior margin instead of the tip.

TABLE 4  
YY S/CY D/1 X Bd

	SD		SD Bd		S		S Bd		Cy D		Cy D Bd		Cy		Cy Bd		Express- sivity	All	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂			
Number.....	89	84	5	19	103	73	4	14	81	72	4	10	82	80	3	7	III-V		
Penetrance..			10.6	36.8			7.4	32.2			9.4	24.4			6.6	16		8.6	28

(5) There is no reason to assume (table 4) that the markers used (Cy, D, S) had in themselves a modifying effect (however, see page 114).

In all these crosses one of the second chromosomes of the Bd stock might have contained Dexter and Muller's enhancer of scalloping, but was not checked. To eliminate this a stock was built up containing the third chromosome with the Bd locus and the other third chromosome and the second

TABLE 5

CHROMOSOMAL COMBINATIONS WITH Bd AFTER REPLACING 1ST, 2D AND 3D OR 2D AND 3D CHROMOSOMES AND OUTCROSSING TO OREGON

Cross	Individuals	Penetrance Bd in per cent	Expressivity class
Control Ore × Bd.....	555	2	III
Control Bd × Ore.....	1536	2	III
yy S/Cy D/Bd × Ore.....	862	18	III-V
Ore × S/Cy D/Bd (from yy).....	777	5 4	III-IV
yy Ore/Cy D/Bd × Ore.....	169	8	III
Ore × Ore/Cy D/Bd.....	228	3 4	III-IV
yy S/Ore D/Bd × Ore.....	230	2 6	III-IV
yy S/Ore D/Bd × Ore.....	985	17 4	III-V
yy S/Ore D/Bd × Ore.....	378	4	III
yy Ore/Cy Sb/Bd × Ore.....	194	6	III
Ore/Cy D/Bd × Ore.....	1520	9 6	III-IV
Ore × S/Ore D/Bd.....	403	3 6	III-V
Ore × S/Ore D/Bd.....	178	6 8	III-IV
Ore × Ore/Cy Sb/Bd.....	106	6	III
Ore × Ore/S Sb/Bd.....	401	6	III
Ore × Ore/S D/Bd.....	691	4	III
Ore × S/Cy D/Bd.....	271	4	III
Ore × Ore/Ore Ore/Bd.....	368	2	III
Ore × Ore/Cy Ore/Bd.....	150	6	III
Bd I/Ore Bd II/Cy Bd/Inv × Ore.....	385	22	III-V
Bd I/Ore Bd II/S Bd/D × Ore.....	363	24	III-V
yy Bd II/Ore Bd/Sb × Ore.....	94	14	III-V
Ore × S/Ore Inv/Bd.....	225	20	III-V
Ore × Ore/Bd II Bd/Inv.....	339	10	III-V
Ore × Bd II/Cy Bd/Inv.....	254	10	III-V

chromosomes, and sometimes also the first, replaced by marked chromosomes. (Bd was crossed to S/Cy D/1 or /Sb, and Bd/D flies were backcrossed to the marked stock with attached X yellow present or absent, and thus the stock obtained was S/Cy D/Bd with or without yy.) Thus many combinations were produced containing the Bd chromosome without the inversion and without the second chromosome from the Bd stock. The replacing chromosomes were either Oregon or chromosomes with dominant markers or combinations of both. The penetrance and expressivity varied in such an irregular degree that no significant difference among these combinations could be stated. Table 5 contains a few examples in which lines containing the Bd chromosome from Bd stock were outcrossed to Oregon to give different heterozygous combinations

of the markers with Oregon chromosomes known to act towards low penetrance. Each cross produced the different combinations that were expected. The individual counts are not listed. The controls have the usual low penetrance, which also occurred where three Bd chromosomes were replaced by Oregon chromosomes. Otherwise the foreign chromosomes had no enhancing or diminishing influence except their generally enhancing effect when compared with Oregon chromosomes. The second chromosome from Bd stock has, however, a clearly enhancing influence.

Chromosomal combinations with Bd, after replacing first, second, and third, or second and third chromosomes and outcrossing to Oregon, are shown in table 5.

TABLE 6  
F<sub>2</sub> WITH BD FROM F<sub>1</sub> TABLE 1

Cross	Classes								Penetrance in per cent		Penetrance F <sub>1</sub> in per cent	
	I/II		III/IV		V		VI					
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
(sy × Bd) <sup>a</sup> . . . . .	612	502	20	20	70	84	67	130	30.6	47.7	27.6	28.4
(ss × Bd) <sup>2</sup> . . . . .	336	307	18	23	21	21	49	31	31.2	33.5	9.4	7.4
(Bd × sy) <sup>2</sup> . . . . .	230	243	22	14	25	28	39	35	40.8	36.1	25.4	30.2

Because of the enhancing influence of the second chromosome from Bd stock, it was of importance to try to isolate the influence of the supposed enhancer in the second chromosome in homozygous condition together with Bd without the inversion. The results obtained by using dominant markers for the second chromosome were insignificant. Data are, however, available for F<sub>2</sub> from the F<sub>1</sub> contained in table 1. When bred from clearly Beaded F<sub>1</sub> parents 2/3 of the F<sub>2</sub> were heterozygous for Bd without the inversion and of these 1/4 have both second chromosomes from the Bd stock. The presence of the enhancer ought to be visible in comparison to the F<sub>1</sub> crosses. Table 6 exhibits the results.

These data may mean that an intensifier exists in the second chromosome, working in the absence of the inversion. Intensifiers from the non-Bd parents may also take part, but probably do not, in view of the similarity of results both for sy and ss, which produce such a different penetrance in F<sub>1</sub>. In all three crosses the increase in expressivity has paralleled that of the penetrance. Expressivity has not been calculated because of a different classification used (classes III and IV united), but a high percentage of individuals of class VI show this parallelism. These data again raise the question whether the inversion has the full effect only in the presence of the second chromosome modifier. To test this the inversion was repeatedly reintroduced in the absence of the second chromosomes from Bd stock. As the inverted chromosome is not marked, the procedure was to cross Bd to Cy/S D/l, backcross, and breed from Cy/S D non-Bd individuals. If no nicked individuals appeared in the following generations the balanced stock was Cy/S D/Inv. This crossed to the

combination yy S/Cy D/Bd produced the Bd/Inv recombination in all non-D flies. In all these crosses the non-D flies were all or almost all Bd, thus showing that the In(3R)C inversion alone is needed for the  $\pm$  all Bd condition of the original stock.

#### INTENSIFIER OR POSITION EFFECT?

According to the classic conception, the action of the inversion opposite Bd is supposed to be based upon the presence of an intensifier within the inversion. Doubts as to the correctness of this interpretation were raised when the senior author ascertained many years ago that In(3R)C could be

TABLE 7  
CROSSES (Sb/PAYNE  $\times$  Bd) INBRED

Cross no.	Non-Bd + or Sb		Bd non-Sb						Penetrance in per cent	
			II/III		IV/V		VI			
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
4720.....	105	98	..	1	5	10	10	3		
4721.....	130	141	..	3	4	1	21	7		
4722.....	52	55	..	..	..	..	9	4		
Total.....	290	294	..	4	9	11	40	14	57.6	36

TABLE 8  
CROSSES BETWEEN Bd AND FLORIDA WILD STOCK

Cross	+		Bd		Class	Penetrance in per cent	
	♀	♂	♀	♂		♀	♂
Bd $\times$ Fla.....	559	373	155	118	II-VI	65.1	72
Fla $\times$ Bd.....	58	33	28	25	II-VI	116.7	129.6
Total.....	617	406	183	143		68.7	78.3

replaced by an inversion that was completely unrelated. Bd was crossed for purposes of marking with different marker stocks, with the results already reported. Among the marker stocks was one containing Stubble (Sb) over the so called Payne Inversions, now known as In(3L)P, In(3R)P, inversions which have breaks different from those of In(3R)C and which certainly have different origin. The expected result was that these inversions largely replaced the enhancing action of In(3R)C, as shown in table 7.

A few other third chromosome inversions were subsequently tested. One result is rather gratifying because the presence of the inversion was not known. In crossing Bd to wild stock Oregon was usually used, resulting in about 2-5 per cent penetrance in the heterozygote, as reported. Crosses with Florida wild stock gave a different result (penetrance is calculated under the assumptions that the compound of two inversions is lethal and that the segregating classes are equal. The expected small percentage of penetrance in flies without the

Only the summarized results are recorded, but the individual broods showed either much higher or much lower penetrance. For example, a group of crosses, not contained in the table, all of which had a high per cent of penetrance, actually had a calculated value of over 100 per cent Bd. Consequently, the Florida stock was crossed into different stocks in which the Bd chromosomes except the one containing Bd were replaced by marked chromosomes, so that a possible interaction of Florida and Bd chromosomes could be excluded. The result was the same, as is shown in table 9.

TABLE 9

THE RESULT OF SUBSTITUTING CHROMOSOMES OF THE Bd STOCK IN THE CROSS Bd  $\times$  FLA

Cross	Total flies	Penetrance Bd in per cent	Class Bd
yy bw/bw e Bd/e $\times$ Fla.....	270	over 100*	II-VI
reciprocal.....	111	over 100	II-VI
yy S/Cy D/Bd $\times$ Fla. ....	62	over 100	II-IV
yy S D/Bd $\times$ Fla.....	941	93	II-IV
Fla $\times$ Cy/+ D/Bd.....	367	over 100	II-IV

\* Percentages over 100 can be based upon the penetrance in the combination Bd/+, or on differential viability of the classes of segregation, or both.

In order to locate the source of the enhancing action, the Florida stock was crossed and backcrossed to marker stocks, thus combining individual Florida and foreign chromosomes. Testing these combinations against Bd it turned out that the high penetrance was only found when the third Florida chromosome took part. A subsequent check of the salivaries by M. Kodani revealed, in most flies of this stock, an inversion identical with In(3R)P. This then, replaces the action of In(3R)C.

Inversions In(3R)C, In-Payne, and In(3R)P, originating respectively in Bd, wild, and Florida stock, could hardly all contain the same intensifying mutant. To minimize such a possibility, two more third chromosome inversions were tested: a (3R)P inversion of still different origin, and an inversion in the left arm of chromosome III, In(3L)P.L.V.M. Also, the In(3R)C was reintroduced from another line balancing Sb over this inversion. This might, however, have been the inversion derived from the Bd stock, as the origin of this balanced stock is unknown. But as the compound of this and the Bd-stock inversions, although less viable, turned out not to be lethal, the two inversions are somehow different although both are homozygous-lethal. This point, irrelevant for this analysis, was not investigated further. The following table contains the results of crosses between Bd and stocks containing different inversions in the third chromosome, penetrance again being calculated for the segregating class containing Bd over the inversion. The positive results are evident in table 10.

By chance, still another combination was obtained. The balanced Beaded stock was crossed to yy S/Cy D/Sb in order to replace all but the Bd chromosome. The marker stock was very poorly viable, and the same was true for the crosses, which yielded only about 20 individuals each. As there were hardly



any Bd phenotypes among these, proper selection was difficult and it became necessary to resort to mass breeding. After a few generations the mixed stock suddenly changed; it became rather viable, the number and degree of beaded flies increased, and individuals appeared which were highly beaded and simultaneously contained all four markers, S, Cy, D and Sb. As Sb remained with Beaded in outcrossing these flies, Sb and Bd had obviously been put into the same chromosome by crossing over. These Star, Curly, Dichaete, Stubble, Beaded flies not only bred true but showed also an almost complete penetrance and viability. The first two inbred pairs produced 177 Beaded and 28 + flies, a penetrance of 86.3 per cent. This increased in further generations to about 001 per cent. In addition, the grade of scalloping was one or two classes higher than

TABLE 10  
CROSSES BETWEEN Bd AND STOCKS CONTAINING DIFFERENT INVERSIONS  
IN THE THIRD CHROMOSOME

Cross	Non-Bd	Bd	Penetrance Bd/Inv in per cent	Remarks
Sb/IN(3R)C × Bd/Inv	77	41	over 100 <sup>a</sup>	59 Sb:59+, 1 Sb Bd
In(3R)C/Ore from Sb stock × Bd, Inv	236	67	88	
D Sb ca <sup>2</sup> /Inv(3R)P × Bd/Inv	912	270	74.4	526 D:656+, i.e., Compound Inv. viable, 26 D Bd 244+Bd
In(3L)P.L.V.M./+ × Bd/Inv	614	184	92	

<sup>a</sup> See table 9.

in the original balanced Bd stock. This condition was thought to be due to crossing over of the In(3R)C into the Dichaete chromosome, made possible by the mass culture. Simultaneously, either at the time when Sb-Bd crossed over or when D-Inv did the same, something that had diminished viability of the crossover flies was removed from the third chromosome of the marker stock. Thus the new combination increased in number and could be easily isolated. The higher grade of expressivity of scalloping would be understood as the result of additive action of the In(3R)C and the Dichaete inversion. The study of the salivary chromosomes actually showed the presence of the inversions, and genetic tests later confirmed the interpretation. For example, yy S/Cy D Inv(3R)C/Sb Bd × Oregon produced the usually low penetrance and expressivity in F<sub>1</sub>, thus showing that the Sb section in the Bd chromosome did not enhance the action of Bd/+ (penetrance 6.7 per cent). Either the new marker stock or F<sub>1</sub> flies with Oregon, but showing D, were crossed to the standard Bd stock, thus producing the combination D-Inv(3R)C/Bd in all phenotypically Dichaete flies. All Dichaete flies were Bd of the highest grade, as expected, all non-D flies, whether Bd or Sb Bd, showing the usually small percentage of Bd (actual penetrance D-Inv(3R)C/Bd = 100 per cent, Bd/+ and Bd Sb/+ = 5.1 per cent in this group of crosses). As D and In(3R)C enhance each other

when Bd is present, the small Dichaete inversion should also be assumed to have an enhancing effect. Crosses to test this gave rather inconsistent results. Some of them have been reported above; another one made simultaneously with the present set of experiments gave 17 per cent penetrance for D/Bd, but without elimination of other possible modifiers. The subject was not investigated further.

#### INTERACTION OF BD AND VG

Dexter determined that the presence of heterozygous vestigial enhances the Bd action. The effect of both loci is similar so far as scalloping is concerned, and also so far as the phenotype of the heterozygote can easily be shifted from recessivity to marked dominance by modifiers. But the pattern of the scalloping differs for the two mutants. (For vg see illustrations in Mohr, 1932; for Bd, see Bridges and Morgan, 1923.) As a rule, the vg effect, in heterozygotes with dominigenes as well as in compounds with alleles, begins as a nick at the tip and continues indenting from the tip, later engulfing the posterior edge. The anterior edge of the wing, at least its proximal part, remains intact up to the highest grades of scalloping (see alleles strap and antlered). Only rarely do other types of scalloping occur. In Bd the first step is a nick either at the tip or at the posterior edge, only rarely at the anterior edge. The next grade consists almost exclusively of scalloping of the posterior wing edge. Still higher grades show the beading of the anterior edge produced by a series of small nicks, followed by greater or less destruction of the anterior edge through scalloping. Braun, in this volume, shows that both phenotypes may be produced experimentally in the vg heterozygote. It was therefore of interest to see how the simultaneous presence of both heterozygous mutants would affect the pattern. In order to eliminate the inversion In(3R)C, which now is known to enhance vg action also (Gardner, this volume), standard vg was crossed to a hybrid Bd  $\times$  ss. Non-Beaded flies of the latter cross could be Bd/ss or Inv/ss; beaded flies were Bd/ss. The following table contains the backcrosses vg  $\times$  (Bd  $\times$  ss). The cultures nos. 4667-4669 are crosses with beaded F<sub>1</sub> hybrids, and nos. 4670-4673 are crosses with not-beaded F<sub>1</sub>. Actually cultures no. 4672 and 4673 turned out to be crosses with ss/Inv. The classification of the individuals is very difficult. Besides normal the following classes were discerned:

(1) The kn series—i.e., the typical vestigial series as described and classified in the senior author's former papers. Class I is a small nick at the tip of one wing and class VI corresponds roughly to Mohr's scalloped.

(2) A series which shows a combination of the vg and Bd effects. SS shows the vestigial scalloped or snipped effect at the tip, but at the posterior edge is typically strong Beaded. A is a type similar to the vg allele antlered but with the anterior margin missing. J is a narrow straplike wing without anterior or posterior margin, similar to the Beadex allele Bx<sup>J</sup>. EV means extra vein.

Thus the penetrance of Bd without inversion in the presence of vg/+ is almost complete. Of the females 159 are not scalloped, 119 scalloped; of the males 126 not scalloped, 122 scalloped. The difference in the females may be the result of a greater viability of females without Bd. The table shows further that most of the females and males exhibit a combination of the patterns of the two mu-

TABLE 11  
VG X (SS X Bd)

Cross no.	+		Classes of kn																			
			I		II		III		IV		V		VI		SS		A		J		Almost vg	EV
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂		
4067 ..	47	34	1	2	-	-	-	1	-	-	-	1	2	11	24	29	7	4	-	-		
4668 ...	25	16	-	1	-	-	-	-	-	-	-	-	2	12	16	11	9	-	-	-		
4669...	42	48	1	-	-	-	-	-	2	-	-	-	2	16	21	17	8	-	-	-		
4670.....	43	28	2	5	-	-	1	4	-	-	-	1	2	9	9	10	5	4	3	1	2	
Total	157	126	4	8	-	-	1	5	2	2	4	1	5	48	70	57	29	8	3	1	2	
4672 .	151	154	9																		8	
4673 ...	35	35	3											1								
Total	186	189	12											1							8	

tants, and only a small group exhibits no Bd effect (the higher kn classes). Further the expressivity of the scalloping, though highly variable, as in the Bd-balanced stock, goes far beyond that known either in our pure Bd stock or in vestigial heterozygotes even in the presence of dominance modifiers, except the facet alleles. Therefore the scalloping process as such is doubtless the same in both mutants; further, the developmental agent which produces scalloping can be increased in its activity by the addition of two different causative agents. But the pattern of scalloping seems based upon different processes, both of which may combine, without giving up their identity. The last two broods show the slight dominance-enhancing effect of In(3R)C upon the females heterozygous for vg.

After the discovery of the dominance modifiers (dominigenes) for vg/+, the question arose, whether they would also act upon Bd in the absence of vg. Only one set of experiments was made for orientation. A Beaded stock in which the chromosomes 1, 2, and 3, except the one carrying Bd, were replaced by marked chromosomes (some without marked X chromosome) was crossed to individuals heterozygous for vg and homozygous for the three dominigenes (see Goldschmidt 1935, 1937). The control crosses were identical except for the presence of a chromosome marked with Sb to replace Bd. The combinations resulting from such a cross are many and different. In the controls half of the offspring are vg/+. This group will be scalloped if the sex-linked dominigene is present in the males, the females being heterozygous. Females without the sex-linked dominigene will not be scalloped, but if this modifier is heterozygous in females, they will be scalloped in part if an enhancing inversion, like D or Cy, is present (see former papers).

The expectations for the experiments then were: one half of the offspring vg/+, one half not; both may combine with Bd, or not; all are heterozygous for the autosomal dominigenes, with the sex-linked dominigene distributed as in the controls. The expected difference in the results is, then: one fourth of both sexes simultaneously contain Bd/+ and vg/+, and are, therefore, scalloped in the higher grades; one fourth of the individuals are Bd/+ without vg/+, which means a small percentage of scalloped flies of the lower grades are produced when the dominigenes do not enhance the Bd action, but more when they do; one fourth of both sexes are vg/+ without Bd, like the controls, and one fourth contain neither Bd nor vg and are normal. Therefore, in the yy crosses, where no kn females can appear in the controls, the presence of more than a little over one quarter of scalloped females would be caused by an interaction of Bd with the autosomal dominigenes. In the absence of yy and in the males of the yy cross the presence of many more scalloped individuals than the percentage of the controls plus one-fourth of all individuals would have to be accounted for by combined Bd and dominigene action. Table 12 contains the data of the controls and experiments. In these experiments, performed many years after most of the earlier ones, the scalloping effect was classified differently. Classes I-VI correspond to the class III-VI of our table 1; classes VII-IX correspond to SS and A in table 11; kn Doho means the homozygous dominigene stock heterozygous for vg and therefore scalloped; kn means scalloped; the Roman numeral shows the class of scalloping.

TABLE 12  
CROSSES BETWEEN FLIES FROM BD-BALANCER STOCK AND KN DOHO FLIES

Cross	S D		S D kn		Cy D		Cy D kn		S Sb (or Bd)		S Sb (or Bd) kn		Cy Sb (or Bd)		Cy Sb (or Bd) kn	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Control 1. yy S/Cy D/Sb × kn Doho. ....	13	8	—	1 I	14	3	—	10 II	9	4	—	5 II	15	7		7 I-II
1. yy S/Cy D/Bd × kn Doho....	3	6	6 VIII	6 III-VII	7	5	3 VIII	2 II	5	6	4 VII-VIII	6 II-VII	13	2	2 VII	5 II-VIII
Control 2. S/Cy D/Sb × kn Doho. ....	17	13	8 II	12 I	11	10	10 I	13 I	17	18		14 I	12	9	10	I-IV
2. S/Cy D/Bd × kn Doho.....	8	4	III-IX	13 I-IX	5	4	14 I-IX	16 I-IX	4	2	13 I-IX	20 I	10	2	11 II-VIII	15 II-VII
Control 3. kn Doho × S/Cy D/Sb.....	19	21	2 II	7 II	25	13	3 II	9 II	20	18	4 II	6 II	15	14	4 II	4 II
3. kn Doho × S/Cy D/Bd.....	4	2	1 VII	9 VII	2	5	8 VII	10 I-VIII	11	4	3 III	4 III	1	5	—	3 I-III

In cross 1 the ratio of nonscalloped to scalloped females is 28:15, expectation about 32:11, which is an insignificant difference. In cross 3, where all males have the sex-linked dominigene and all females are heterozygous for it, the ratios are: ♀ ♀, 18:12; ♂ ♂, 16:24. As *vg* dominigene action requires the sex-linked dominigene in homozygous (or simplex) condition, we would expect an effect only in the males. Actually many more scalloped males are found than the expectation derived above, but the numbers are unfortunately small as all these crosses have a poor viability. Finally, in cross 2 the ratios are: ♀ ♀, 27:49; ♂ ♂, 12:64. Compared with the controls this might indicate a dominigene interaction with *Bd*, but in view of the high percentage of *kn* in the controls, we do not feel sure. The problem does not seem important enough to warrant the use of more complicated methods of analysis.

TABLE 13  
CROSSES WITH Bx F<sub>1</sub>  
CLASSES LIKE TABLE 1

Cross	♀			♂ all
	Flies	Class	Penetrance Bx/+ per cent	
1. sy × Bx.....	117	III	2.	+
2. sy × Bx .....	244	....	0	+
3. sy × Bx.....	209	III, IV	25.4	+
4. sy × Bx.....	49	IV, V	35.3	+
5. sy × Bx.....	73	IV	84.2	+
6. Bx × sy.....	210	....	0	Bx
7. Bx × sy.....	73	IV	26.7	Bx
8. Lausanne × Bx.....	150	III	4.	+
9. Lausanne × Bx.....	132	III	8.9	+

#### INTERACTIONS WITH BEADEX

Beadex, a sex-linked dominant, is another well-known locus producing scalloping. It is viable in homozygous condition and the phenotype is somewhat different from that of Beaded. There are no normal or nicked individuals in the homozygous stock and the range of variability is rather small, as opposed to *Bd*. If a classification were used similar to that for *Bd* in table 1, all females would belong to class VI, all males to class V. In the stock which the senior author used, the anterior and posterior edges of all female wings were clipped. The *Bd*-like series of indentations at the posterior edge was replaced by a straight clipping. Only rarely was the wing tip nicked, as in *Bd*. Most males had a normal anterior margin, and the clipping of the posterior edge was more *Bd*-like, with indentations and notches.

There were two points of interest in connection with the present work: (1) Did *Bx*, like *vg* (and also *fa*, as a few tests showed), enhance *Bd* action, and (2) is *Bx* action also enhanced by the presence of *In(3R)C*? We must first know the amount of dominance or the penetrance of scalloping in the *Bx* hetero-

zygote. As table 13 shows, the penetrance in heterozygous females varies considerably, indicating a dependence upon modifiers, which were different in the nonisogenic stocks used for testing. The  $F_2$  (table 14) actually shows that selection for such modifiers is possible, and an analysis similar to that for the *vg* dominigenes could be carried out if such a repetition were of interest.

Actually  $F_2$  from  $F_1$  with low penetrance was again low (no. 1, 26);  $F_2$  from scalloped  $F_1$  individuals in high penetrance crosses (no. 4, 5) had high penetrance;  $F_2$  from non-scalloped individuals, much lower penetrance. The *sy* stock then obviously carried dominance modifiers in different combinations, as had already been noticed in the *Bd* crosses, the same modifiers apparently

TABLE 14  
CROSSES WITH  $Bx \times F_2$   
CLASSIFICATION AS IN TABLE 1. CLASS VII WINGS ARE STRAPLIKE

$F_1 \text{ } \varphi$	$\varphi$						$\sigma$		Penetrance per cent $\varphi \text{ } Bx/+$
	+	III	IV	V	VI	VII	+	$Bx$	
No. 1 +.....	67	..	..	1			32	26	3
No. 1 +.....	121	..	..	..			48	54	0
No. 1 +.....	108	..	..	..			22	32	0
No. 1 +.....	131	..	..	..			57	45	0
No. 4 class IV.....	96	..	16	12			62	51	45.1
No. 4 +.....	10	..	1	..			12	12	18.2
No. 4 +.....	153	..	12	5			87	71	10.0
No. 6 +.....	74	..	..	20		70	88	48	9.7
No. 6 +.....	72	1	..	5		71	66	60	3.4
No. 5 class IV.....	98	..	14	11			60	61	40.6
No. 7 class III.....	55	..	1	30	35		64	39	10.0
No. 7 +.....	71	..	7	26	66		91	75	16.5

acting with both loci. As was previously stated, this situation thus parallels closely that studied for the dominance of vestigial and was therefore not further analyzed.

The eventual combination effect of both heterozygous loci  $Bx/+$  and  $Bd/+$ , as well as the eventual effect of the Inversion upon  $Bx$  expressivity and penetrance, can be read directly from the results of a cross of a female  $Bx \times$  male  $Bd$ , both from the tested stocks. The daughters of such a cross are all heterozygous for  $Bx$  and half heterozygous for  $Bd$ , half for the Inversion. They show, therefore, the combination effect of both heterozygous dominants as well as the possible effect of the inversion upon heterozygous  $Bx$ , both in comparison with heterozygous  $Bx$  and  $Bd$ . The males are all  $Bx$ , otherwise like the females, and thus show the interaction of  $Bd$  as well as the inversion with  $Bx$  simplex. Actually such an  $F_1$  contains only scalloped flies. The females are mostly in class IV, some in classes V and VI. The males go far beyond the scalloping in either  $Bd$  or  $Bx$ . They belong partly to what has previously been termed class VII, having narrow straplike wings clipped at both edges; a large number of them have still narrower wings, like a slender strap, almost identical with the

type of the  $Bd^J$  allele, a type that might be recorded as class VIII. Whatever the penetrance of  $Bd$  or  $Bx$  would have been separately in the heterozygote, it was raised to 100 per cent for  $Bx/+$  in combination with the  $Bd/+$  as well as with the  $Inv/+$  third chromosomes, and the expressivity of  $Bx$  in the males (already with 100 per cent penetrance) was much increased both by  $Bd$  and the inversion.

Further generations were bred to obtain the other possible combinations. In  $F_2$ ,  $Bx/Bx\ Bd/+$  females were obtained which had extreme straplike wings, class VIII, as expected. In later generations, also, the combination  $Bx/Bx\ Bd/Inv$  was synthesized, which showed in part, probably in the presence of another modifier in the second chromosome, vestigial-like wings. Although they appeared in a majority of the individuals, they could not be maintained true breeding. The analysis was not carried beyond this point.

### DISCUSSION

The facts reported may appear at first sight to be just another contribution to the problem of dominance modifiers. A closer analysis, however, reveals that basic problems of structure and action of the germ plasm are hidden behind the facts. If the whole body of data could be completely understood, important and fundamental insight might be gained. Thus far a satisfactory synthesis has not been possible. Therefore, only a few points may be emphasized.

All inversions in the third chromosome act as enhancers of penetrance and expressivity of the  $Bd$  mutant locus located in the homologous chromosome. The one inversion tested had the same effect upon the  $Bx$  heterozygote and also upon the homozygote— $Bx$  in the first chromosome,  $Bd$  in the third—showing that a problem not of dominance, but of intensity of action, is presented. Gardner (this volume) showed that all inversions in all chromosomes tested had an enhancing effect of the same type upon the vestigial heterozygote, but only when the three invisible dominance modifiers were also present. These three and the inversions act here like a system of multiple factors with special threshold conditions. Green and Oliver (1940) have recently presented facts which point in the same direction. Such results reveal a type of position effect which is comparable to the effect of enhancers and dominance modifiers. They show that chromatin rearrangements, although without visible effect, nevertheless have definite effects if the proper conditions are fulfilled. The genetic action of these position effects then is the same as that of so-called invisibles among mutants, which must be supposed to comprise most mutants: invisibility of the effect in a definite genetic milieu does not mean absence of effect. This must be kept in mind in consideration of the relation between position effect and mutation (see also Goldschmidt, Gardner and Kodani, 1939).

The facts presented in this paper reveal further remarkable relations. In a paper by the junior author (Gardner, this volume) it was shown that the same enhancing effect upon scalloping is also produced by the presence in heterozygous condition of the mutant facet, whether or not it works in conjunction with the dominigenes upon the  $vg$  heterozygote. The same applies to



the Notched deficiencies and to a facet allele without visible effect. Facet is a mutant characterized by an eye effect, but it simultaneously produces nicked wings with a low penetrance. It is, therefore, generally assumed that the Notched deficiencies which include the facet locus owe their scalloping to the  $+^{fs}$  locus, which in simplex condition cannot control complete wing development. We shall not now challenge this interpretation. The action of facet as enhancer of  $vg/+$  may be the result of two additive incomplete scalloping effects. But Goldschmidt found what seems to be an allele of facet without any visible effect, but which has an extreme effect upon the dominance of  $vg$ . For details see Gardner (this volume). Similarly, at the cut locus the sex-linked dominigene for  $vg/+$  is, so far as can be ascertained, an allele of cut, but has no visible action. It can be replaced by cut: in a female the compound  $ct/ct^{do-vg}$  has exactly the same effect as  $ct^{do-vg}$  homozygous. But  $ct$  causes scalloping which in itself, without the dominigenes, enhances the  $vg/+$  action only very little if at all. To this may be added from the present paper a corresponding interrelation between  $vg$ ,  $Bd$ ,  $Bx$ ; from former papers by Czik and Waletzky, other interactions with  $Bx$ . Waletzky demonstrated that with bifid and  $Bx$  simultaneously present the action is not simply additive but disproportionately large, true also of the increase of  $vg$  action by our facet allele. This is certainly a complex set of interrelated facts, which cannot be, as yet, understood altogether on the basis of a simple principle, especially when the problem of pattern is added to the rather simple problem of scalloping.

The direction in which a solution may be sought is perhaps indicated in the following summary: scalloping of  $vg/vg$  can be more or less suppressed by action of sublethal temperatures (Roberts, *et al.*). Scalloping in Beaded is greatly influenced by environment (Dexter, *et al.*). Wild type pupae treated at a critical period with temperature shocks develop into scalloped flies (Goldschmidt). The same is true of wild type larvae treated with X rays (Friesen). The degree of scalloping in  $vg$  alleles (W. Braun) and the degree of dominance of  $+$  over  $vg$  (Gardner) can be shifted regularly by changes in developmental speed. The shift of dominance in heterozygotes of  $vg$  and of phenotype in homozygous vestigial and alleles exhibits strange and complicated temperature relations (Harnly, *et al.*). The specific pattern of scalloping found in the  $vg$  series can be experimentally modified into the different pattern of the  $Bd$ - $Bx$  series (Braun).

The facts just summarized hold out the hope that all data presented here will some day be integrated and understood in terms which must necessarily be rather simple. One interpretation which has been presented before in terms of speeds of differentiation, also discussed by Green and Oliver, will not be repeated, as it has been sufficiently discussed by Goldschmidt (1938). It may be stated only that scalloping by histolysis can be produced and enhanced by such a series of genetic and nongenetic agencies (recessive and dominant loci, modifying loci, position effect of inversions and translocations, triploidy, trisomy, additive action of unrelated loci as well as of alleles of one locus, temperature, temperature shocks, X rays) that a condition of the threshold type must be supposed to be back of it. A rather simple way of shifting such a condition, say by shifts in relative speeds of integrating processes, may be expected to furnish

the explanation. The facts relating to the pattern suggest that the same type of explanation will account also for the problems of pattern involved.

### SUMMARY

(1) Study of expressivity and penetrance of *Bd* in Muller's balanced stock, and of the heterozygous *Bd* locus freed from the intensifiers in the stock and in different combinations, showed that mean expressivity was always proportional to the degree of penetrance. The penetrance varied from a small percentage to over 30 per cent in certain combinations, and revealed that a sex-linked enhancing action was present in the *Bd* stock. The stock *sy* always contained an autosomal dominant enhancer; Oregon did not.

(2) Some observations were made on the character *extra vein*.

(3) The enhancer found by previous authors in the second chromosome has a definite action in the absence of the inversion, but the action cannot be discerned in its presence.

(4) We endeavored to learn whether the huge intensifying action of the inversion opposite *Bd* is due to an intensifier therein or by a position effect of the inversion. As three different inversions, some of them in samples of different origin—*In*(3R)C and *In*(3R)P from Payne, Florida and Pasadena stocks, *In*(3L)P.L.V.M. and Payne—could replace each other, the intensification would seem ascribable to position effect. *In*(3L)D and *In*(3R)C together with *Bd* give the maximum effect in penetrance and expressivity.

(5) *Bd* without inversion is, alone, hardly effective; *vg* heterozygous, alone, is normal. Together, however, they produce a huge scalloping effect of a very high grade of expressivity. The two scalloping actions increase each other in an exponential way. The pattern of scalloping shows a combination of the patterns typical for each mutant.

(6) The dominance of *vg* is also enhanced by *In*(3R)C from *Bd* stock.

(7) A test of whether the dominigenes for *vg* action also enhance the dominance of *Bd* gave no fully convincing result.

(8) *Beadex* interacts with *Bd*, as *vg* does, differently in the sexes. *Beadex* homozygous further enhances the additive action, and the addition of the inversion from the *Bd* stock produces *vg*-like flies. Also, the penetrance and expressivity of *Bx* are enhanced by the inversion.

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A STUDY OF HEREDITARY HOMOEOSIS: THE  
MUTANT TETRALTERA IN DROSOPHILA  
MELANOGASTER

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# A STUDY OF HEREDITARY HOMOEOSIS: THE MUTANT TETRALTERA IN DROSOPHILA MELANOGASTER

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THE TERM "HOMOEOSIS" was introduced by Bateson (1894) to denote the change of one organ of segmental series from its own characteristic form to that of another in the series. This phenomenon is important not only for the genetics of the particular species involved but also for the light it casts on problems of comparative anatomy and evolution.

Homoeosis has long been known from cases of abnormal regeneration—occurring naturally or produced experimentally, of an antenna in place of an eye, of a leg in place of an antenna, and so on. Inherited homoeotic changes are found in many orders of arthropods. Lebedinsky (1925) cites the lepidopteran family Zygaenidae in which the metathoracic wings have become identical to the mesothoracic wings. In the aberrant dipteran *Termitozenia*, which lives commensally with termites, the wings are much reduced and intermediate between wings and halteres (Wasmann, 1900). Bezzi (1916) reported that in a number of Hymenoptera, Neuroptera, Strepsiptera, and Coccids the metathoracic wings are reduced to appendages resembling halteres, while in parasitic Hymenoptera, Orthoptera, and Homoptera, the mesothoracic wings may be halterelike.

A number of homoeotic mutants in *Drosophila melanogaster* have been found and studied. These include bithorax (3-58.8) and bithorax-b (3-58.8+) (Bridges and Morgan, 1923), tetraptera (3-51.3) (Astauroff, 1927, 1929), aristapedia (3-58.5) (Balkashina, 1929; also found in *D. simulans* by Sturtevant, 1929), proboscipedia (3-47.7) (Bridges and Dobzhansky, 1933), and two cases of the homoeotic appearance of an antennalike structure in place of an eye or in combination with a rudimentary eye (Goldschmidt, 1929, 1940; Gottschewski and Ma, 1937; Valadares, 1938).

All these homoeotic mutants share certain characteristics which distinguish them more or less from other mutants:

(1) The phenotype is highly variable: individuals that are genetically identical may differ much in external appearance. These variations in phenotype may be arranged in a series ranging from a normal, unchanged organ through many intermediates to a complete or almost complete homoeotic replacement by a homologous organ.

(2) There are great variations in the percentage of individuals that show the character at all, although all of them are genetically homozygous for the mutant factor. Many will appear completely normal. Thus, the "penetrance" (Timoféeff-Ressovsky 1926) of the character is variable, rarely reaching 100 per cent in most homoeotic mutants. Variations in penetrance result from various chance environmental factors, such as temperature, moisture, and food.

(3) The symmetry of the expression of the character varies greatly. Complete symmetry is extremely rare; most individuals are asymmetrical to a greater or less degree. Many individuals are completely normal on one side, but show the mutant character on the other.

(4) A low degree of right-left correlation exists in the expression of the character. Astauroff (1930) made a statistical analysis of his data on tetraptera and concluded that the appearance of the character on one side was independent of its appearance on the other. Symmetrical individuals appear as a result of the accidental coincidence of two equal and independent probabilities.

Goldschmidt (1940) pointed out that in all of these homoeotic organs, apart from their special features, there is the general characteristic that the homoeotic organ has primarily the appearance of a palpus—a primitive appendage consisting of a few segments which may specialize into an antenna, a tarsus, or a haltere. The series of intermediate forms of these organs, which appear in the various homoeotic mutants, show that the basic difference between the various appendages is the type of segmentation which occurs in their development. An explanation of the action of these mutants in determining homoeotic changes was derived by Goldschmidt (1938) from Balkashina's data on the development of the mutant aristapedia; this explanation is discussed later in this paper.

The mutant "tetraltera," which is described in this paper, is a typical example of a homoeotic change. In it the normal mesothoracic wing is replaced by a halterelike appendage, with a large series of intermediate forms. The extreme expression of the character produces a fly with four halteres, hence the name "tetraltera." Tetraltera resembles the other homoeotic mutants in that it is variable in its expression, has a low penetrance or overlaps the wild type in a large percentage of cases, and has a low left-right correlation. Inasmuch as the morphology of the character and of its series of intermediates was of the highest interest, a detailed morphological study was made and is presented herein.

I wish to express my gratitude to Professor Richard Goldschmidt, who suggested this problem and furnished much invaluable advice and criticism throughout its progress. Assistance rendered by personnel of Work Projects Administration, Official Project 65-1-08-113, Unit C-1, is gratefully acknowledged.

In 1934 Goldschmidt discovered the tetraltera mutant in two crosses of *Bd* ♀ × *Bd* ♂. The parental *Bd* flies came from the same *Bd* × *sy* cross. Twelve tetraltera flies (6 ♀ ♀ and 6 ♂ ♂) appeared in one cross (no. 4435 in Dr. Goldschmidt's records), and three tetraltera, all males, in the other cross (no. 4436). From these flies a stock of tetraltera was established and brought to Berkeley in 1936. When the mutant first appeared, the phenotype was fairly regular. The wings were spoon-shaped, with a thick outer ring of tissue and a depressed, thin region in the center, resembling the tetraptera phenotype (pl. 10, fig. 5). After some generations this type was replaced by one in which the wing was normal in shape but in which the veins showed many abnormalities. Still later the present condition of great variability of phenotype appeared.

## MORPHOLOGY OF TETRALTERA

## DESCRIPTION OF TETRALTERA PHENOTYPES

The mutant tetraltera is extremely variable in its phenotypic expression and therefore a complete description of all types that may be found is impossible. Only the most interesting and typical members of the series of phenotypes will be described, although an almost continuous series of intergrades exists. For comparison with these tetraltera types, the normal wing and haltere are described.

The normal wing (pl. 10, fig. 1) is an oval-shaped structure, about 2.5 mm. long, 1 mm. wide at the widest point. It has five main longitudinal veins: the costal or marginal at the anterior outer margin; then, in order posteriorly, the radius, medius, cubitus, and analis. There are two cross veins: the anterior, between the medius and cubitus, and the posterior, between the cubitus and analis. There is also a small, incomplete sixth longitudinal vein to which the anal cross vein attaches.

The normal haltere, or balancer (pl. 10, fig. 2) is a small club-shaped appendage about  $350\mu$  long, on the metathorax over the metathoracic spiracle, just where the metathoracic wing is attached in other orders of insects. It is usually turned upward, outward, and backward. The haltere is made up of three parts: a basal segment which bears complicated plates on which are sensory papillae; a stalk which is rather short; a head, on which there is a deep horizontal gash on the posterior, inner side; below this gash is a group of bristles. The rest of the surface of the head, stalk, and base is covered with fine hairs.

The penetrance of the tetraltera mutant is about 15 per cent under normal culture conditions; that is, only about 15 per cent of the flies homozygous for the character are changed at all; all the others are completely normal. The character may appear only on one side or on both sides, and, if on both, the two may be quite different. Completely symmetrical individuals occur only rarely.

The various phenotypes produced by the tetraltera genotype may be classified into four groups, three based on changes in the wing structure, and one on changes in the mesothorax itself. In the first group the wing may be reduced in size, altered in shape, or have parts missing or doubled, but it is still essentially winglike, bearing veins. In the second group the mesothoracic appendage is a halterelike structure, in which the three-segmented condition of the appendage is more or less clear. There is a complete series of sizes of these structures from 2 mm. down to about  $500\mu$ . In the third group of phenotypes the mesothoracic appendage is still further reduced to a small stump, a single segmented palpus, or is completely missing. The fourth group exhibits changes in the mesothorax which occur simultaneously with some change in the wing. These usually occur with a class II or III wing, rarely with a class I, and never with a normal wing on the same side of the body.

The class I changes may be divided into four main types, all of which are essentially winglike. The first of these, type Ia (pl. 11, fig. 10), is a wing in



which the posterior part, including the analis vein, does not develop, and the wing is curved inward and backward. In certain more extreme members of this class, both the analis and cubitus veins are missing.

The second class I type, Ib (pl. 11, fig. 11-13), is a curious wing in which an abnormal growth of the inner proximal surface occurs, together with a whole set of abnormal veins in this region, which suggests that a doubling or splitting of the wing disc has occurred in development. These new veins are connected to the analis vein.

Type Ic (pl. 11, figs. 14-17), are wings which are large, saclike and filled with fluid, resembling the phenotype of the mutant "inflated."

Besides these three main categories, many other changes in wing structure occur, although less often; these, grouped together as type Id (pl. 11, figs. 18-19), include wings which are almost normal in shape and venation but smaller in size, "blistered" wings which have large bubbles in the wing blade, and many other abnormalities in venation and shape.

The class II phenotypes are the most interesting forms, because they approach the shape and structure of a haltere. A continuous series of types occur, varying from a large, saclike structure resembling a "balloon" or "inflated" wing, to a clearly three-segmented, halterelike structure with a base, stalk, and head. For convenience, this group has been divided arbitrarily into four types.

Type IIa (fig. 1a-c) includes phenotypes at the less halterelike end of the series, in which the outer part of the wing appendage is a large saclike structure up to 2 mm. long. This "head" of the mesothoracic appendage is oval in shape, with two rows of bristles running longitudinally on the dorsal side of the sac, one at the anterior margin, the other at the posterior margin, and meeting at the distal tip of the head. In some cases the area between these two rows of bristles is depressed so that the center of the appendage is much thinner than the edges. The division of this appendage into segments is usually not clear.

Type IIc (fig. 2a-e) consists of the appendages at the more halterelike end of the series, in which the mesothoracic appendage is clearly three-segmented, with a proximal base, a thin stalk, and a distal, enlarged, spheroidal head. The bristles on this head may form a circle (fig. 2b), be restricted to one or two rows at its distal end (fig. 2d), or be few in number and scattered over the distal end in a manner suggesting the bristle pattern of a haltere (fig. 3f).

Type IIb includes the transition forms between types IIa and IIc (figs. 1d-f, 2a). In some appendages, one or two large bristles occur on the very tip of the head, which from their size, position, and curved shape suggest the claws on the ends of the leg segments.

Type IId are phenotypes in which the mesothoracic appendage is a simple three-segmented palpus (figs. 2e-f, 3a). The base and stalk are similar to the corresponding parts of type IIc, but the head or distal segment is not enlarged and spheroidal as in IIc, but small and palpuslike, without large bristles and with small bristles distributed evenly over its surface.

In the class III phenotypes the wing is reduced to a small stump or may even be completely missing. In type IIIa (figs. 3b-c) the stump remains as a

one- or, rarely, two-segmented, simple palpus covered with fine hairs and small bristles. It usually has several large bristles at its base and may have a few large bristles at the distal end of the palpus. In type IIb, when the appendage is absent, the mesothorax in the region where the wing should be attached is smooth, showing no trace of the wing. No hairs, microchaetes, or bristles grow in this region.

Class IV includes changes in the mesothorax itself rather than in the mesothoracic appendage. These changes occur only in flies in which the wings, too, are altered. Usually the wings belong to class II or III; rarely do these changes occur with class I. These changes in the mesothorax are not correlated with the wing changes but are completely independent; no particular change occurring in the mesothorax is always associated with a particular wing type. Since these changes in the mesothorax are quite different, two or more of them may occur in the same fly on the same side; they are not mutually exclusive as are the changes in the wings.

The first of these changes in the mesothorax, IVa, consists of hairy outgrowths of various sizes on the posterolateral edges of the mesonotum, posterior to the supra-alar bristles and posterior and lateral to the postalar bristles (figs. 1a, 2c-d, 3a-b). The outgrowth may be large, longer than a normal scutellum (fig. 3b), or small, consisting merely of a small, hairy bump (fig. 1a). While smallest outgrowths are covered only with small bristles, the larger ones have bristles as large as the supra-alar bristles. The larger outgrowths are frequently divided into two almost equal parts by a deep notch in the border and a groove down the center. The large bristles do not form any regular pattern on most of these outgrowths and cannot be recognized as being homologues of particular bristles on the normal mesothorax. However, the outgrowth is sometimes developed enough so that it can be recognized as a half-thorax, a complete lateral half of a mesonotum, including the scutellum. Thus the dorsal mesothoracic disc appears to have split or doubled, forming two half-thoraxes on one side, one normal in size, the other small. The notch and groove on the other outgrowths in this type become the line dividing this extra half-thorax from its scutellum.

The type IVb flies have one or more extra bristles on the scutellum (figs. 1b, c, e, f, 2e, f, 3d). Usually only one extra bristle is present, growing lateral to the normal anterior scutellar bristle on a small outgrowth from the side of the scutellum, suggesting a partial doubling of the scutellum. The scutellum is occasionally changed to a greater degree and several extra bristles are present. In figures 1c, e, f, the left anterior scutellar is doubled and another extra bristle occurs lateral to this on a lateral outgrowth from the scutellum. In other flies the scutellum is so changed that the fundamental bristle pattern is difficult to recognize; thus, in figure 2a probably the right half of the scutellum is missing and the two bristles are the left anterior and posterior scutellars, whereas in figure 3d the remaining bristles cannot be homologized with any of those normally present.

In another type of change in the mesothorax, IVc, the scutellum is missing. This change may occur only on one side so that half the scutellum is missing

(figs. 1*d*, 2*a*, 3*c*, *d*), or on both sides, so that the whole scutellum is missing (figs. 2*b*, 3*e*). These figures demonstrate that the scutellum, as well as the rest of the mesonotum, has a double source; each side of the body develops from its own disc independently of the other side. Thus, in figure 3*f* the left side of the thorax grew almost normally, though slightly altered in position and shape, even though the right side was completely missing because of the failure of the right mesothoracic disc to evert. When the scutellum is completely missing (figs. 2*b*, 3*e*), the posterior part of the mesothorax is smoothly rounded and bears no large bristles. In some flies the microchaetes in this region form a whorl (fig 2*b*).

Many changes occur in the bristle pattern of the rest of the mesonotum which have been classified together as type IV*d* (figs 1*e*, 2*b*, 3*c-d*). Frequently, when the scutellum is absent, the dorsocentrals will be missing. Of course, the scutellar bristles are absent whenever the scutellum is gone. The supra-alars and postalars are often missing or changed in class II or class III flies, and also may be absent when the scutellum is absent. The humerals, notopleurals, and presutural bristles are changed or missing least often. These bristles are altered only after large changes have occurred in the whole mesothorax. As in the scutellar bristles, the bristles on the mesonotum are sometimes difficult to homologize and name when large changes in it have occurred.

Type IV*e* is a further change in the mesonotum, beyond absence of scutellum and changes in bristle pattern, in which the dorsal mesothoracic discs fail to complete their growth dorsally and medially and therefore do not meet in the dorsal midline (fig. 3*e*). The area in the midline between the discs is filled in with undifferentiated tissue which has no hairs or microchaetes. In these flies the bristle pattern is greatly changed and difficult to correlate with the normal one. In some flies only one of the mesothoracic discs fails to complete development, whereas the other side of the mesonotum is more or less normal (fig 3*c*). This again shows the independence, in their development, of the two sides of the thorax.

In the final step in the reduction of the thorax, type IV*f*, the dorsal mesothoracic disc never everts. This results in hemithorax flies (fig. 3*f*) when only one of the discs fails to evert, and in "no-thorax" flies when both fail to evert. In these flies the ventral part of the thorax and the mesothoracic legs develop normally, but the dorsal mesothorax is almost wholly lacking, so that the head and abdomen are drawn close together. No hemithorax fly has ever been found with a normal wing on the side of the body which lacks the mesonotum, although hemithorax flies with class II or class III wings are known. The "no-thorax" females are apparently sterile.

These phenotypes produced by the tetraltera genotype can be arranged in a more or less continuous series running from a normal wing through reductions of parts of it (I*a*) to a saclike expanded form filled with body fluid (I*c*), on through a gradual change of this sac to a smaller three-segmented, halterelike structure (II*a*, II*b*, and II*c*). This structure may be reduced first to a three-segmented palpuslike structure (II*d*), to a two- or one-segmented palpus (III*a*), and finally may be altogether missing (III*b*). The mesonotum and other parts of the dorsal mesothorax may be affected simultaneously with these

changes, so that first the scutellum is missing (IVc), next the bristles of the mesonotum are deranged and deleted (IVd), then the dorsal mesothoracic discs fail to complete their development and to meet in the dorsal midline (IVe), and finally the dorsal mesothoracic disc may completely fail to evert (IVf). In addition to the foregoing there are interesting types which suggest a doubling of the various parts of the mesothorax: Ib in which the wing or perhaps a part of it is doubled; IVa in which the mesonotum or some part of it is doubled; IVb in which the scutellum is doubled.

#### COMPARISON OF TETRALTERA WITH TETRAPTERA

The striking similarities between the tetraltera and tetraptera series of phenotypes show that the same or similar developmental processes are affected, sometimes in the same way, sometimes in the opposite way by these two mutants. The smallest change in the tetraltera wing-haltere series, type Ia (pl. 11, fig. 10), resembles the high tetraptera type (pl. 10, fig. 7). In tetraptera the last part of the wing to appear is the posterior part, including the analis and cubitus veins; in tetraltera, the first part of the wing to be changed is this same posterior part, and the analis and cubitus veins are the first to disappear.

The figures show an interesting similarity and difference between these two mutants. On the inner medial border of the tetraptera metathoracic appendage (pl. 10, fig. 6) is an outgrowth resembling in position and shape the outgrowth in tetraltera type Ib (pl. 11, figs. 11-13). In the next highest tetraptera type (pl. 10, fig. 7) this outgrowth becomes a small club-shaped appendage, resembling a double haltere, whereas in tetraltera it becomes a flat structure with veins, resembling a double wing.

The intermediate stage in both tetraptera and tetraltera (fig. 4a and pl. 11, figs. 14-17) is the inflated bag or balloon stage, called Ic in the tetraltera series. This bag may show veins in tetraltera but not in tetraptera.

In the next step in the series the head of the appendage is an oval structure with thick edges and a thin center. In tetraltera there are two rows of bristles along the two thick edges, one on each edge, which meet at the distal end of the appendage (type IIa, figs. 1a-c). In tetraptera the corresponding stage has bristles only at the distal edge of the appendage (pl. 10, figs. 4 and 5).

In the most halterelike members of the two series (pl. 10, fig. 3; fig. 2b-d) the appendage is similar to a normal haltere, with no differentiation of the head into a thick "edge" and thin "center"; instead, the head is a homogeneous spheroidal structure with only one or two rows of bristles at the distal edge of the appendage. Some of the IIc tetraltera appendages are almost exact halteres, and the most modified tetraptera appendage is an almost exact wing (pl. 10, fig. 8).

In addition to these similarities of the wing-haltere series are others:

(1) The haltere in tetraptera or the wing in tetraltera is absent in a small percentage of flies; not the slightest trace remains of the appendage.

(2) In tetraptera a small outgrowth covered with hairs and bristles may develop on the dorsal part of the metathorax (pl. 10, fig. 9), and in tetraltera a similar outgrowth occurs on the posterolateral border of the mesothorax (figs.

1a, 2c, d, 3a-b). This indicates that both mutants affect the development and differentiation of other parts of the dorsal meso- and metathoracic discs than the wing and haltere buds.

#### COMPARISON OF TETRALTERA TYPES WITH THE WINGS OF TERMITOXENIA

The remarkable taxonomic parallel to the tetraltera mutant provided by the aberrant dipteran *Termitoxenia* was pointed out by Goldschmidt (1940). There are several genera and a number of species of this fly, all living commensally with termites in Ceylon, Java, other East Indian islands, and Africa. These flies have minute rudimentary wings (fig. 4a-d) which are incapable of flight. The wings have been transformed into structures which may be specific sense organs intermediate in shape and size between wings and halteres. When the flies were first discovered, Wasmann (1900) and his followers believed that these appendages were not wings or wing homologues. Subsequent work has indicated, however, that they are wing homologues and Kemner (1940), reviewing the controversy, refuted the views of Wasmann and his school.

Kemner pointed out that the wings of the different members of the *Termitoxenia* group are built on the same general pattern and may be divided into two or three types. All the wings consist of a costa, a radius, and a small cubito-analis part, but differ in the relative size and shape of these parts.

In one type, such as *Termitomyia mirabilis* (fig. 4d), the distal third or fourth of the costa is bent under and backward, lying behind the point of the radius. The radius is distended and blistered but little, or not at all. Similar to this type are the wings of *Ptochomyia afra*, *Termitoxenia gracilis*, and *Termitostroma schmitzi*.

Another distinct type is that in which the costa of the wing is not bent at the end but lies on a plane with the radius and is more or less pointed on the end (fig. 4a, c). The outer one-half to two-thirds of the radius is often strongly distended and blistered and has several small clear pores. This type of wing, occurring in the genera *Clitelloxenia*, *Javanoxenia*, and *Odontoxenia*, and in *Termitoxenia heimi*, is thus characteristic of the oriental species, whereas the first type appears in the African forms.

A third, intermediate, type, *Termitoxenia jagerskioldi* (fig. 4b), is similar to the second type but differs in that the end of the costa is bent slightly over the radius and is rounded rather than pointed at the end.

Kemner thinks that the first type is the most primitive, showing the greatest similarity to the hypothetical ancestor of *Termitoxenia*, which most probably belonged to the Phorida. The wing of *Termitomyia* can be interpreted as a remnant of a phoridan wing strongly reduced in the posterior part. The radius is little changed and contains small bars of chitin which occur in many normal Phorida. *Termitomyia* is also more primitive in its head and abdominal structure than is *Javanoxenia*. Kemner thinks that this reduction of the *Termitoxenia* wing from the phoridan wing may have occurred in a single step. He mentions the phoridan *Echidnophora butteli*, whose wings are strongly developed and possibly functional in early life but later break along a definite

line behind the radius, leaving a remnant consisting of a costa, radius, and a small cubitoanalis part, much like the wing of *Termitoxenia*.

All the wing homologues of *Termitoxenia* are intermediate between wings and halteres; none resemble a true haltere closely, since they are not segmented and do not have a distinct "head," and the distribution of the bristles along the costa is more or less winglike rather than haltere-like. They are not entirely winglike since they are not flat and do not have veins. They do resemble one of the intermediate members of the tetraltera wing-haltere series, type IIa, much more than they resemble either a wing or a haltere. If one compares the *Termitoxenia* wing, especially the second or third type mentioned, with a tetraltera type IIa (figs. 1a-c), the essential similarities are readily seen. The anterior dorsal part of the IIa appendage bearing the large bristles could be called the costa—it actually is homologous to the costa of the wing, inasmuch as a complete series of intermediates in both tetraptera and tetraltera connect this row of bristles in this stage with the costal vein and its row of bristles in the normal wing—and the distended, sac-like part of the appendage posterior to the costa could be called the radius. Hence the *Termitoxenia* structure and this intermediate tetraltera type, which also occurs in the tetraptera series, correspond part for part; both lack the posterior part of the normal dipteran wing.

## INHERITANCE OF TETRALTERA

### HOMOZYGOUS NATURE OF THE TETRALTERA STOCK

When the tetraltera stock was received from Dr. Goldschmidt in August, 1938, it showed a penetrance of 15.3 per cent, there being 116 tetraltera flies in the total of 756. The stock has been maintained by inbreeding brother to sister in standard laboratory half-pint culture bottles on the usual cornmeal-agar-molasses food seeded with fresh yeast. In January, 1939, the stock was divided into 25 lines, each of which has been inbred since, for more than 45 generations. But no increase in the penetrance of the character, or decrease in the variability of its expression, has occurred. No significant differences appear among the penetrances of the various inbred lines. The average penetrance of them all has remained at about 15 per cent. The penetrance in different culture bottles ranges from 1–45 per cent.

These single-pair matings were made: phenotypically normal ♀ × phenotypically normal ♂, phenotypically tetraltera ♀ × phenotypically tetraltera ♂, phenotypically tetraltera ♀ × phenotypically normal ♂ and phenotypically normal ♀ × phenotypically tetraltera ♂ from the tetraltera stock. All gave the same results: the penetrance in the offspring was the same whether tetraltera or normal flies from the tetraltera stock were used in breeding. This indicates that the tetraltera stock is homozygous for the tetraltera locus and that tetraltera, like many other mutants, is manifested in only a certain percentage of the flies homozygous for it. Whether or not the character appears in any given fly probably depends upon some threshold condition affected by chance environmental conditions, such as temperature, moisture, or food.

Crosses of tetraltera with various wild stocks—Canton, Oregon, and Florida—and with a number of other mutants showed that it was recessive to the normal condition; it did not appear in the heterozygous condition. When the  $F_1$  flies from the Oregon  $\times$  tetraltera cross were inbred, 8 tetraltera flies appeared in  $F_2$  among 3125, corresponding to a penetrance of one per cent for homozygous flies. A possible explanation of this low penetrance is given on page 139.

#### LOCALIZATION CROSSES WITH BALANCED LETHAL STOCKS

Crosses with attached-X stocks showed that tetraltera was not sex-linked, so crosses were made with the S/Cy, D/Sb "balanced lethal" stock to determine the chromosome in which tetraltera was located. Single-pair matings of tetraltera  $\times$  S/Cy D/Sb were made and the  $F_1$  male Cy D flies were back-crossed to tetraltera females. The offspring of this cross included: 423 Cy D, 324 Cy, 462 D, 269 normal, 59 tet, 1 Cy tet, and 11 D tet. Tetraltera was later located in the third chromosome; therefore this cross may be symbolized:

$$\frac{+ \text{tet}}{+ \text{tet}} \text{ } \varnothing \times \frac{\text{S D}}{\text{Cy Sb}} \text{ } \sigma$$

$F_1$ :

$$1 \frac{\text{S D}}{+ \text{tet}} : 1 \frac{\text{S Sb}}{+ \text{tet}} : 1 \frac{\text{Cy D}}{+ \text{tet}} : 1 \frac{\text{Cy Sb}}{+ \text{tet}}$$

$$\frac{+ \text{tet}}{+ \text{tet}} \text{ } \varnothing \times \frac{\text{Cy D}}{+ \text{tet}} \text{ } \sigma$$

Back-cross offspring ratio:

$$1 \frac{\text{Cy D}}{+ \text{tet}} : 1 \frac{\text{Cy tet}}{+ \text{tet}} : 1 \frac{+ \text{D}}{+ \text{tet}} : 1 \frac{+ \text{tet}}{+ \text{tet}}$$

The appearance of both Curly tetraltera and Dichaete tetraltera flies in this cross could mean that:

(1) The tetraltera gene is located in the fourth chromosome (later disproved by crosses with fourth-chromosome mutants).

(2) There are two or more factors, one in chromosome II and one in chromosome III, either of which could produce the tetraltera phenotype (disproved when tetraltera was located in the third chromosome).

(3) These are examples of occasional crossing over in the male parent.

(4) Dichaete may change the dominance of tetraltera so that a fly heterozygous for tetraltera appears phenotypically tetraltera. As other dominigences for tetraltera were found later, this last explanation is most probable.

The cross was repeated, using Gla/Cy D/Sb, with similar results.  $F_1$  males back-crossed to tetraltera females gave 844 Cy or Gla, D or Sb; 540 Cy or Gla; 927 D or Sb; 662 normal; 60 tetraltera; 1 Cy tetraltera and 7 D tetraltera. These results confirm those of the previous crosses and show that there is some common explanation for them and that they are not due to experimental error.

This cross was repeated later using only Star and Stubble, mutants which had not shown either enhancing or suppressing effects upon tetraltera. Tetraltera was crossed to Star Stubble flies, and then F<sub>1</sub> Star Stubble males were back-crossed to tetraltera females. In the F<sub>2</sub> six classes appeared: Star Stubble, Star, Stubble, normal, tetraltera, and Star tetraltera. There were 38 tetraltera and 43 Star tetraltera flies among the 5500 offspring. The appearance of tetraltera with Star and not with Stubble proves conclusively that tetraltera is in the third chromosome.

#### CROSSES WITH FOURTH CHROMOSOME MUTANTS

To test the possibility that tetraltera was located in the fourth chromosome, crosses were made with ey, cy-D, and Scn. The tetraltera stock used was one in which the mutants striped (allele of ebony, 3-70.7) and glassy (allele of glass, 3-63.1) had arisen.

Eyeless females were crossed to striped glassy tetraltera males and in the F<sub>1</sub> 6 male tetraltera flies appeared among the several hundred normal flies. This meant either that tetraltera was an allele of eyeless or that eyeless or some factor from the cycless stock acted as a dominigene to tetraltera. The F<sub>1</sub> flies were inbred and a large F<sub>2</sub> generation (21,345 flies) obtained of which only the tetraltera flies were counted:

CLASSES	♀ ♀	♂ ♂
tetraltera .....	17	41
tetraltera striped .....	0	2
tetraltera glassy .....	3	1
tetraltera striped glassy .....	5	22
eyeless tetraltera .....	5	8
eyeless tetraltera striped .....	0	0
eyeless tetraltera glassy .....	1	0
eyeless tetraltera striped glassy .....	2	3

Since tetraltera appears in this F<sub>2</sub> group in combination with eyeless it cannot be an allele of it; therefore the appearance of the tetraltera flies in the F<sub>1</sub> must result from some dominigene action of the cycless locus or of some other locus in the cycless stock.

When the foregoing F<sub>2</sub> counts are rearranged according to tetraltera non-eyeless and tetraltera eyeless classes, a close approximation is obtained to the 3:1 ratio expected on the basis that tetraltera and eyeless are in different chromosomes. Thus tetraltera is not located in the fourth chromosome.

CLASSES	OBSERVED	EXPECTED
tetraltera .....	58	53
tetraltera eyeless .....	13	18
tetraltera striped .....	2	1.5
tetraltera striped eyeless .....	0	0.5
tetraltera glassy .....	4	3.75
tetraltera glassy eyeless .....	1	1.25
tetraltera striped glassy .....	27	24
tetraltera striped glassy eyeless .....	5	8



The consistent smallness of the eyeless classes is probably due to a lower viability of eyeless flies or perhaps to incomplete penetrance of the eyeless character.

As tetraltera was later located in the third chromosome at locus 48.5, it is linked to striped and glassy, and therefore the striped glassy tetraltera class (the noncrossover class) should have been largest. The marked excess of tetraltera over striped glassy tetraltera, and of tetraltera eyeless over striped glassy tetraltera eyeless, must be due to the dominigene action of the eyeless locus or some factor from the eyeless stock. Some of the 58 tetraltera flies produced above must have been genotypically  $tet/+ +/ey$ , and some of the 13 tetraltera eyeless flies must have had the genotype  $tet/+ cy/ey$ .

Three tetraltera eyeless males appeared in the  $F_1$  of crosses between eyeless-Dominant females and striped glassy tetraltera males, showing that eyeless-Dominant has the same dominigene action as eyeless. Eyeless males from this  $F_1$  generation were backcrossed in single-pair matings to striped glassy tetraltera females. Among the 86½ offspring were 2 ♂♂ striped glassy tetraltera, 8 ♀♀ and 20 ♂♂ striped glassy tetraltera eyeless, and 2 ♀♀ and 8 ♂♂ tetraltera eyeless. When the  $F_1$  tetraltera eyeless males were backcrossed to tetraltera females, 140 offspring were produced of which 1 ♀ and 1 ♂ were striped glassy tetraltera, 4 ♀♀ and 6 ♂♂ were striped glassy tetraltera eyeless, and 1 ♀ and 3 ♂♂ were tetraltera eyeless. The appearance of these tetraltera eyeless flies shows again that the eyeless-Dominant factor or some factor in the eyeless-Dominant stock acts as a dominigene to tetraltera. Inasmuch as both eyeless and eyeless-Dominant show this dominigene effect, and only eyeless tetraltera and never non-eyeless tetraltera flies were produced in the eyeless-Dominant crosses, the eyeless locus itself, rather than some other locus in these stocks, probably has this dominigene action.

Some of the  $F_1$  eyeless females were backcrossed to striped glassy tetraltera males, and among 395 flies in the offspring there were the following: 1 ♂ striped glassy tetraltera, 3 ♀♀ and 3 ♂♂ striped glassy tetraltera eyeless, 3 ♀♀ and 1 ♂ tetraltera eyeless, 1 ♂ tetraltera 1 ♂ tetraltera striped eyeless, and 1 ♂ tetraltera striped. Of these, the striped glassy tetraltera and striped glassy tetraltera eyeless are definitely noncrossover classes; the tetraltera, striped tetraltera eyeless, and striped tetraltera are definitely crossover classes; and the tetraltera eyeless may or may not be crossover products, depending upon whether the genotype is  $tet + +/tet gl^4 e^{st}; ey^D/+$ , which would be a crossover product, or  $+ + +/tet gl^4 e^{st}; ey^D/+$ , a noncrossover product.

In these three crosses the ratio of 65 eyeless tetraltera flies to 7 non-eyeless tetraltera is much greater than the 2:1 ratio expected. This suggests that eyeless-Dominant increases the penetrance of tetraltera when the tetraltera factor is homozygous in addition to acting as a dominigene when tetraltera is heterozygous. This evidence is particularly clear in the backcrosses of male  $F_1$  eyeless flies to female striped glassy tetraltera, where the expected offspring ratio is 1 striped glassy tetraltera to 1 striped glassy tetraltera eyeless. Instead, discounting the tetraltera eyeless class, the ratio was 4 striped glassy tetraltera

to 38 striped glassy tetraltera eyeless. The eyeless recessive locus does not show this penetrance-increasing action.

To prove that  $ey^D$  was acting as a dominigene the following test was made. Tetraltera flies were crossed to  $ey^D$  and then  $F_1$  eyeless flies were crossed to Sb. 3 ♀♀ and 5 ♂♂ eyeless Stubble tetraltera flies appeared in the offspring of this cross among 2500 flies. The tetraltera factor must be heterozygous in these flies because the other third chromosome contains the Stubble factor, so that the appearance of phenotypically tetraltera flies must result from the dominigene action of  $ey^D$ .

Scutenick females were crossed to striped glassy tetraltera males and  $F_1$  Scutenick males were backcrossed to striped glassy tetraltera females. No tetraltera flies appeared in the  $F_1$ , and none in the  $F_2$  which were not also striped and glassy, showing that Scutenick does not have the dominigene action of eyeless and eyeless-Dominant.

#### NEW MUTANTS: THEIR CROSSES WITH TETRALTERA

Several new mutants arose in the tetraltera stock in the course of this investigation, some of which were used in localizing the tetraltera factor itself. A recessive mutant causing curled wings appeared in November, 1938, but owing to its low viability was lost before it could be localized. In August, 1939, a recessive mutant causing a plexate (extra veins in the wing cells) condition appeared. This was localized in the second chromosome and is an allele of plexus (2-100.5). Another recessive mutant causing dumpy or truncate wings, which had appeared twice before but had been lost each time, reappeared in January, 1940 and has been localized in the third chromosome near the Beaded locus.

The recessive mutant striped appeared in November, 1939. This mutant causes the trident on the dorsal side of the thorax to be heavily pigmented, giving three clear longitudinal stripes which meet at the anterior edge of the scutellum. It was found to be an allele of ebony (3-70.7) and has been given the symbol " $e^{st}$ ."

The latest mutant to appear was glassy, in April, 1940. Glassy reduces the size of the eye by one-half, changes the appearance of the facets to a fused sheet, and reduces the eye color. The eye is completely white in the female and a faint pink in the male. Glassy proved to be a more extreme allele of glass (3-63.3) in which the facets are rough and the eye color is pink in both sexes. The new glassy mutant has been given the symbol " $gl^4$ ."

A stock containing striped, glassy, and tetraltera was prepared and used to localize tetraltera. This stock was outcrossed to Canton-S wild type and the  $F_1$  females were backcrossed to striped glassy tetraltera males using single-pair matings. The backcross offspring totaled 9455, of which 5 ♀♀ and 33 ♂♂ were tetraltera; 65 ♀♀ and 107 ♂♂ were striped glassy tetraltera; 8 ♀♀ and 10 ♂♂ were glassy tetraltera; and 2 ♀♀ and 4 ♂♂ were striped tetraltera. These figures give a crossover value of  $14.53 \pm 2.30$  per cent between glassy and tetraltera and  $26.4 \pm 2.88$  per cent between striped and tetraltera, which locates tetraltera at about locus 48.5 in the third chromosome.

In another series of crosses similar to those with striped and glassy, tetraltera showed a crossover value of 32.2 per cent with rough and 41.9 per cent with claret, placing it about at locus 58 in the third chromosome. Since tetraltera is so far from both rough and claret, many double crossovers must have occurred so that tetraltera is really farther to the left in the third chromosome than is indicated by these crossover values. The estimate of 48.5 obtained with glassy is more accurate since glassy is nearer to tetraltera.

Moiré, a dominant factor associated with a large inversion in the left arm of the third chromosome, was found to have the same dominigene action with tetraltera that Dichaete, eyeless, and eyeless-Dominant have. Star Stubble Moiré females were crossed to tetraltera males and the  $F_1$  Star Stubble Moiré males were backcrossed to tetraltera females. In the  $F_1$  some Stubble Moiré tetraltera and some Star Stubble Moiré tetraltera flies appeared; and in the  $F_2$ , among about 1500 flies, there were 2 ♀♀ tetraltera, 1 ♀ and 4 ♂♂ Star tetraltera, 2 ♀♀ and 3 ♂♂ Stubble Moiré tetraltera, and 1 ♀ and 8 ♂♂ Star Stubble Moiré tetraltera. The appearance of tetraltera in the  $F_1$  with Moiré and the foregoing  $F_2$  ratio shows that Moiré changes the dominance of tetraltera so that flies heterozygous for tetraltera appear phenotypically tetraltera.

#### CROSSES WITH OTHER MUTANTS FOR POSSIBLE ALLELISM

After tetraltera had been localized in the same general region of the right arm of the third chromosome that contains so many other homoeotic mutants, crosses were made with each of these available: bithorax, bithorax-Dominant, aristapedia, proboscipedia, and kidney, but no evidence of allelism was found. In each cross the  $F_1$  generation was wild type (except with bithorax-Dominant, where the  $F_1$  showed bithorax) and the  $F_2$  generation flies were either wild type, tetraltera, or the other mutant. No crossover flies—ones showing both tetraltera and the other mutant—were found among 2500 flies of each type of cross examined, showing that tetraltera is closely linked to the other homoeotic mutants, though it is not an allele of any of them. Tetraltera may possibly be an allele of tetraptera but this cannot be tested, since the tetraptera stock has been lost.

The mutants pink (3-48.0), blistery (3-48.7), and maroon (3-49.7) were also tested for allelism to tetraltera, with negative results.

#### CYTOLOGICAL STUDY OF SALIVARY GLAND CHROMOSOMES

The larval salivary gland chromosomes were studied for chromosome aberrations. An inversion was found with breakage points apparently identical to those of In3RC, band 92F, and band 100F, which is lethal when homozygous (fig. 5a). The inversion loops were present in about two-thirds of the preparations; the bands of the chromosomes which did not show loops were in completely normal order. In addition, a heterozygous deficiency for band 87E3 (fig. 5b) and a heterozygous translocation or transposition at band 98C1 (fig. 5c) were found in about two-thirds of the preparations, both in those which showed the heterozygous inversion and those which did not. The band that is transposed into region 98C1 resembles closely the band which is deficient in

region 87E3; possibly a single band was transposed from this one locus to the other; however, this cannot be proved because many other bands of this size and character are scattered through the chromosomes. About half the preparations from heterozygous tetraltera larvae showed these three aberrations. This would be expected since the aberrations are heterozygous in the homozygous tetraltera stock. The presence of this inversion, which is lethal in the homozygous condition, explains the low penetrance in the  $F_2$  of the cross with Canton (see page 134). Some of the tetraltera flies that should have appeared contained this homozygous inversion and therefore died.

None of these aberrations correspond to the genetic locus of tetraltera, so they are probably not related directly to the production of the tetraltera phenotype. All lie to the right of the tetraltera locus. Mutants have not as yet been localized at definite bands in this chromosome; however, band 87E3 corresponds to a genetic region of about locus 60 to 65, and band 98C1 corresponds to a genetic region of about 100. The part of the chromosome that should contain the tetraltera locus, bands 82-85, was studied carefully but showed no aberrations.

## EXPERIMENTAL CHANGES IN ENVIRONMENT AND THEIR EFFECT UPON TETRALTERA

### THE EFFECT OF TEMPERATURE UPON THE PENETRANCE OF HOMOZYGOUS TETRALTERA FLIES

Since temperature affects the penetrance of many mutants, the penetrance of tetraltera was measured at 29°, 25°, 20°, and 14° C.

In the first experiment 10 pairs of parents were placed in each of 22 bottles and allowed to lay eggs for 24 hours. The parents were unselected flies; that is, mixed tetraltera and normal, taken from tetraltera stock bottles. After the parents were removed, the bottles were left in a constant temperature incubator regulated at 29° C. for 9 days. Two of the bottles did not produce any flies; the flies from the other 20, counted on the ninth, eleventh, and thirteenth day, included 3 female and 7 male tetraltera and 1328 normal, a penetrance of less than 1 per cent.

At 25° C. the penetrance of tetraltera is about 8 per cent. One of the localization experiments made at this temperature before the effect of temperature upon tetraltera penetrance was known produced 80 tetraltera and 662 normal flies, a penetrance of 8.3 per cent.

The penetrance of tetraltera varies around 15 per cent at room temperature (20° C.), at which most of the crosses with tetraltera were made. In one count, 740 tetraltera and 3799 normal flies occurred, a penetrance of 16.3 per cent; another count showed 655 tetraltera and 4137 normal flies, a penetrance of 13.7 per cent.

In testing the effect of low temperature upon the penetrance of tetraltera, 3 series of 9 bottles each were prepared. Ten pairs of flies selected at random from tetraltera stocks were placed in each bottle and allowed to lay eggs for 24 hours. Two series, A and C, were placed in the cold room (14.4° C.) im-

mediately after the parents were removed; the other series, B, was kept at room temperature for 24 hours and then placed in the cold room. The flies were removed from the cold room 38 days later. Some flies of series A and B, but none of series C, had hatched at this time. The series A flies were counted on the forty-first day and showed 20 per cent tetraltera. Of the series B flies, 4 per cent of those counted on the thirty-eighth day and 14 per cent of those counted on the forty-seventh day were tetraltera. The series C flies were counted only on the forty-seventh day; 36.7 per cent of them were tetraltera (515 tet and 888 normal). Individual cultures varied from 22-62 per cent. In

TABLE 1  
EFFECT OF LOW TEMPERATURE UPON PENETRANCE

Group	Hours of development before cold shocks	Hours of cold shock	Number of normal	Number of tetraltera	Penetrance (in per cent)
I.....	30	41	782	17	2.1
II.....	67	48	675	25	3.5
III.....	71	45	636	20	3.0
IV.....	92	44	753	31	4.0
V.....	116	48	821	33	3.7
VI.....	143	46	445	18	3.9
VII.....	163	50	871	49	5.3
A <sup>a</sup> .....	30	192	843	89	9.5
Control.....	...	...	1416	63	4.2

<sup>a</sup> Hatched three days later than the others.

addition to showing a greatly increased penetrance, series C flies were generally more extreme in their manifestation of the phenotype. Many hemithorax flies appeared in these cultures.

The penetrance of tetraltera, therefore, has an inverse relationship with temperature, being highest at low temperatures and lowest at high temperatures. The opposite condition obtains in tetraptera in which Astauroff (1930) found a penetrance of 0-1 per cent at 17° C., and a penetrance of about 35 per cent at 25° C.

#### AN ATTEMPT TO FIND A TEMPERATURE-EFFECTIVE PERIOD IN TETRALTERA DEVELOPMENT

Inasmuch as cold increased the penetrance and expression of tetraltera, the question arose whether this increase was caused by the effect of cold on some sensitive period in the development of the fly, or by the great prolongation of the whole time of development, which perhaps gave certain embryological processes a longer time to act.

To answer this question the following experiment was performed. In each of 50 culture bottles, 10 pairs of tetraltera flies were left for 24 hours to lay eggs. The bottles were then divided into 9 groups: one group of 8 was kept at room temperature as controls, another group of 7 was kept at 14° C. for 8 days, and

the rest were divided into groups of 5 and given 48-hour cold shocks (14° C.) starting on successive days. The results are summarized in table 1 and show that there is no sensitive period to cold shocks at 14° C. The control flies and the flies given 2-day cold shocks all began hatching on the eleventh day, but the flies given an 8-day cold treatment did not begin to hatch until the fourteenth day. The generally low penetrance of all the stocks was probably caused by the high temperature of the room during the experiment. The flies in group A which had been left in the cold for 8 days showed a significantly higher penetrance than any of the cold-shock groups. This shows that it is the general re-

TABLE 2  
EFFECT OF HIGH TEMPERATURE UPON PENETRANCE

Group	Hours of development before heat shock	Hours of heat shock	Number of normal	Number of tetraltera	Penetrance (in per cent)
I.....	24	12	21	1	5.0
II.....	36	15	39	3	7.0
III.....	51	13	45	4	8.0
IV.....	66	14	38	2	5.0
V.....	80	11	14	1	7.0
VI.....	92	24	65	4	6.0
VII.....	115	25	100	3	3.0
VIII.....	140	23	55	5	9.0
IX.....	164	23	71	2	3.0
Control.....	...	..	1416	63	4.2

tardation and prolongation of development induced by continued cold temperature, and not the effect of a cold shock at some sensitive period in development, that causes the increase in the penetrance of tetraltera after prolonged cold treatment.

A series of heat-shock experiments was run concurrently. In this, 45 bottles were prepared as above, 10 pairs of tetraltera laying eggs for a period of 24 hours. The bottles were then divided into 9 groups of 5 each and given 12-24 hour heat shocks (35° C.) on successive days. Many of the flies were killed by the treatment, but a few in each group hatched, enough to show that there is no sensitive period to this type of heat treatment. The results are summarized in table 2. The same control flies could be used for both heat- and cold-shock experiments because the flies all came from the same tetraltera stock bottles and, the experiments being concurrent, were under identical conditions on the shelves when not undergoing heat or cold shocks. These heat-shock experiments do not show any sensitive period for the suppression of tetraltera penetrance, either, so the decrease in penetrance observed when tetraltera are raised at high temperature is presumably due to the general acceleration of development, at this temperature and the consequent decrease in the time available for certain embryological processes necessary for the production of the tetraltera phenotype.

# EFFECT OF PARTIAL STARVATION UPON THE PENETRANCE OF TETRALTERA

Larval age at beginning of treatment	Day of hatching																				
	10			11			12			13			14			15			Total		
	+	tet	Per cent	+	tet	Per cent	+	tet	Per cent	+	tet	Per cent	+	tet	Per cent	+	tet	Per cent	+	tet	Per cent
96.....	22	1	3.5	14	1	6.6	4	1	20												
35-90.....																					
35-85.....				12	0	0	47	1	2	46	2	4	0	1	50						
43-60.....							21	0	0	42	2	4.5	79	4	5	8	3	27	150	9	5.6
24-48.....							17	1	5.5	38	4	9.5	13	3	18.7	3	2	40	71	10	12.3

## EFFECT OF CHANGES IN FOOD UPON PENETRANCE

Since the foregoing temperature experiments indicated that a general prolongation and retardation of development increased *tetraltera* penetrance, the effect of other methods of prolonging development were studied.

It had been shown by Child and Albertowicz (1937) and Child (1939) that the addition of a small percentage of Nipagin (methyl parahydroxybenzoate) to the normal laboratory food delayed hatching for several days and affected certain embryological processes involved in the development of the vestigial phenotype. To test the effect of Nipagin on the penetrance of *tetraltera*, 10 bottles were prepared with 0.2 per cent Nipagin added to the normal cornmeal-agar-molasses food; 10 other bottles containing the normal food without Nipagin served as controls. Ten pairs of parents were left in each bottle for 24 hours and then removed. The flies were raised at 23° C. The flies on normal food began to hatch on the twelfth day; those on normal food plus Nipagin, on the fourteenth day. The flies from the Nipagin bottles were counted on the fourteenth and sixteenth days; on the fourteenth day they varied from 0.7–8.8 per cent *tetraltera* in different cultures, and on the sixteenth day from 4.5–24.7 per cent *tetraltera*. A grand total of 171 *tetraltera* and 1140 normal flies hatched from the Nipagin bottles, a penetrance of  $13.04 \pm 0.92$  per cent. The control flies had a penetrance of  $9.6 \pm 0.66$  per cent, there being 230 *tetraltera* and 2151 normal flies. Thus, Nipagin has a slight effect in raising the penetrance of *tetraltera*. This increase in penetrance is not caused by the Nipagin directly, however, but is the result of a prolongation of development produced, in turn, by poor food conditions. The increase in penetrance is approximately what might be expected. A 3-day prolongation of development produced by an 8-day exposure to cold (series A of table 1) raised the penetrance 5.5 per cent, to about double the control penetrance, whereas here the Nipagin treatment raised the penetrance 3.4 per cent after a 2-day prolongation of development.

A second method of prolonging the development of *Drosophila* is the partial starvation of the larvae (Beadle, et al., 1938; Khouvine, et al., 1938; Braun, 1939). This is accomplished by removing larvae at known ages from the normal cornmeal-agar-molasses food and placing them in vials containing agar-agar, glucose, and 10 per cent peptone solution made up according to the Beadle and Ephrussi formula. This partial starvation prolongs development from 1 to 4–5 days, depending upon how early in development the change from normal food is made.

In our experiments, larvae of five different ages were used: 96, 85–90, 65–85, 48–60, and 24–48 hours. The results are in table 3.

As controls, other larvae from the same bottles were left to develop on the normal food. These began to hatch on the ninth day and were counted on the twelfth day. There were 646 normal and 54 *tetraltera* flies in this control group, a penetrance of 7.71 per cent. The results given in the table would seem to indicate that partial starvation does have an effect upon the penetrance of *tetraltera*, that the greater the delay in hatching, the higher will be the penetrance of the *tetraltera* phenotype. However, as will be shown, the penetrance



of tetraltera is greater in flies hatching later even when no treatment is given to the larvae. In this partial starvation experiment 372 normal and 27 tetraltera flies appeared, a penetrance of 6.8 per cent compared with a penetrance of 7.7 per cent for the controls. It is apparent, therefore, that in these experiments partial starvation of the larvae had little or no effect upon the penetrance, probably because the treatment was begun after the tetraltera locus had produced its effect, whatever that may be. The tetraltera wing disc is visibly different from the normal at 72 hours of larval development; the developmental processes producing the tetraltera phenotype must therefore be started well before this time. It will be remembered that only when the cold treatment was begun immediately after the parents were removed, before the larvae had hatched from the eggs, was a large increase in the penetrance produced, indicating that the tetraltera factor acts extremely early in development. This would explain why the Nipagin treatment, acting at this early stage, can produce an increase in penetrance, whereas partial starvation with peptone food, which acts only later, cannot. In the present experiment, larvae treated at 24-48 hours of development showed a slightly higher penetrance than the other groups (disregarding the 85-90-hour group, where the figures are too small to be significant), perhaps indicating that the 24-48-hour period is nearer the time of action of the tetraltera factor.

#### COMPARISON OF THE TIME OF HATCHING OF TETRALTERA WITH NORMAL

In the tetraltera stock bottles it could be observed that the phenotypically tetraltera flies hatched later than the phenotypically normal flies. To compare their total time of development and also to compare the total time of development of phenotypically normal tetraltera flies with that of wild-type flies, the following experiment was devised. Tetraltera flies were crossed to Stubble flies and then  $F_1$  males were backcrossed to tetraltera females, using single-pair matings with 12-hour egg-laying periods. The offspring from this cross were counted on each day of hatching. At the same time single-pair matings of Canton  $\times$  Canton wild-type flies were made, also with 12-hour egg-laying periods, and kept at room temperature beside the backcross matings. In the backcross bottles, since crossing-over is suppressed in males, only three types of flies appeared: Stubble, normal (genotypically tetraltera), and tetraltera. The hatching time of the Stubble flies serves as a control for that of the phenotypically normal tetraltera flies, and the hatching time of the Canton flies serves as a control for that of the Stubble flies. The room temperature was rather high during this experiment, averaging  $26^\circ\text{C}$ ., so that both the experimental flies and the Canton controls began to hatch on the ninth day. The simultaneous hatching of the Stubble and the Canton flies shows that the time of development is the same in both, and makes it possible to call the time of development of Stubble flies "normal" in comparing it with that of tetraltera flies. The counts of Stubble, normal, and tetraltera flies (table 4) show that the development of the phenotypically tetraltera takes considerably longer than normal, and the development of the phenotypically normal tetraltera is delayed in comparison with the control (Stubble) flies. This is especially clear

when the percentages of the various types of flies hatching on each day are considered. Thus on the first day, instead of 50 per cent Stubble and 50 per cent non-Stubble flies, as would be expected if all three types had an equal time of development, the flies hatching were 80 per cent Stubble and 20 per cent normal; no phenotypically tetraltera flies hatched on the ninth day. On the tenth day about equal numbers of Stubble and phenotypically normal tetraltera flies hatched; on the twelfth day 15 per cent were Stubble, 50 per cent normal, and 35 per cent tetraltera. Thus, the time of development of the phenotypically normal tetraltera flies is about 1 day longer than that of Stubble and wild-type flies; that of the phenotypically tetraltera flies is 2-3 days longer than that of Stubble.

TABLE 4  
RELATION OF PENETRANCE TO HATCHING TIME OF TETRALTERA FLIES

Day of hatching	Stubble		Phenotypically normal		Phenotypically tetraltera		Total	Per cent Sb	Per cent +	Per cent tet
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂				
9.....	85	52	20	16	0	0	173	79.2	20.8	0.0
10.....	90	96	70	83	1	2	342	54.3	44.7	1.0
11.....	4	11	11	17	1	3	47	31.9	59.5	8.6
12... ..	1	1	4	3	1	4	14	14.2	50.0	35.8

The table also shows that in both stocks the females develop faster than the males. This has been known to be true for practically all *Drosophila* stocks tested, and may help to explain the fact that tetraltera has a higher penetrance in males than in females.

There were 12 tetraltera flies out of a total of 236 non-Stubble flies in the foregoing experiment, a penetrance of 5 per cent. This is about the expected penetrance for tetraltera at 26° C. There were a total of 236 non-Stubble (phenotypically normal plus phenotypically tetraltera) and 376 Stubble flies instead of the 1:1 ratio expected, an indication that tetraltera flies are less viable than normal stocks.

#### TEST FOR EARLY TEMPERATURE-EFFECTIVE PERIOD

Experiments to determine the temperature-effective period showed that no sensitive period to cold or heat shocks existed at any age between 30 and 160 hours. The results of the embryological study, however, suggested that the tetraltera locus had its effect early in development and the cold-treatment experiment indicated there might be a sensitive period in the first day of development. Eggs placed in cold immediately after laying gave an adult penetrance of 36.7 per cent (series C), whereas eggs kept at room temperature for 24 hours and then placed in the cold until the imagoes hatched gave a penetrance of only 14 per cent (series B). A second test was made for a temperature-effective period during the early hours of egg and larval development. Large numbers of flies were left in bottles to lay eggs and then removed after 2 hours,

thus timing the development of the flies accurately. Cold shocks of 15° C. were given, but the results (table 5) indicate that there is no sensitive period to 15° C. cold shocks during this early age. The penetrances of all the treated cultures are slightly higher than in the controls, possibly because of the slight prolongation of development produced by the treatment. For the cultures with the highest penetrances are those which were in the cold longest; those in the cold a short time have a lower penetrance, no matter at what time after egg-laying the treatment was applied. Therefore, the penetrances of series B and series C

TABLE 5  
EFFECT OF EARLY COLD SHOCKS UPON THE PENETRANCE OF TETRALTERA

Age at beginning of treatment (in hours)	Age at end of treatment (in hours)	Normal	Tetraltera	Penetrance and standard deviation
Control. ....	Control	60	3	5.0±2.7
0.....	11	29	2	6.4±1.2
0.....	18	54	8	13.0±4.2
0.....	24	34	2	5.5±3.7
0.....	30	50	9	15.2±4.6
6.....	19	36	6	14.3±5.3
6.....	24	41	6	12.8±4.9
12.....	20	54	4	6.9±3.3
15.....	39	63	5	7.3±3.1

flies differ, not because of any sensitive period in the first day of development, but because the total time of development of series C flies was much longer than that of series B.

### EMBRYOLOGICAL STUDY

In a study of a homoeotic mutant, it is important to know just when the development of the animal can be seen to change from the normal to its homoeotic course. Balkashina (1929), studying aristapedia, found that segmentation of the antennal disc of the normal fly begins in the 4-4½-day larva, just before pupation, whereas segmentation of the antennal disc of aristapedia begins much earlier, in the 2-day larva, soon after the beginning of segmentation in the leg disc. When segmentation of the normal antenna begins, the aristapedia antennal disc has already divided into 5 segments. In the 3-hour pupal stage, normal antennae have reached their definitive 3-segmented condition, whereas the aristapedia antenna is made up of 7-8 segments. Further development of the normal antenna consists of an extension of the third segment and a differentiation of this into the arista. In the aristapedia appendage, further development and differentiation is typically leglike: the segments become cylindrical, with a thick covering of chitin, the bristles and hairs develop in a tarsuslike pattern, claws and pulvilli form on the end of the last segment. From this, Balkashina concluded that the effect of the aristapedia locus in development is to stimulate the imaginal disc of the antenna to segment at an earlier developmental stage, namely, when the leg disc is beginning to segment, and to guide the differentiation in the direction of a tarsus and not an antenna.

An explanation of the action of homoeotic loci in producing this type of change was derived by Goldschmidt (1938) from the development of arista-pedia as described by Balkashina. Goldschmidt suggested that the pattern of the tarsus in the leg discs, laid down by a process of rhythmical subdivision of the distal end of the anlage, is produced by some kind of evocator, which is in the germ and diffuses into the anlagen of the segmented appendages. Any disc in the proper stage of development will react to this induction by tarsus formation. Normally, only the leg discs are at a proper stage to react to this induction; the antennal disc is far behind in differentiation at this time and is not influenced by the evocator. Chen (1929) observed that the antennal bud is not identifiable as a separate part of the cephalic complex until after 48 hours of larval development. The aristapedia locus, which speeds up the early differentiation of the antenna, makes the antennal disc mature at the same time as the leg discs, and the evocator substance, which causes the formation of tarsus segments, therefore acts in this disc, too. Here, then, a simple shift in the time element of a gene action results automatically in a complicated morphogenetic change. It is not the aristapedia mutant itself which causes the development of a tarsus on the antenna; the aristapedia mutant merely accelerates the early differentiation of the antennal disc so that it can respond to the tarsus evocator, present in all normal flies, which acts at that stage in development. The differentiation of a tarsus on the antenna is controlled by the evocator, not by the aristapedia locus itself.

A search of the literature revealed the following facts about the development of the wing, haltere, leg, and antennal discs. Chen (1929) found that the wing bud develops from the central part of the dorsal mesothoracic disc during the latter part of the fourth day of larval development; the first 5 hours of the prepupal period are critical for wing formation. The wings are everted at about the sixth prepupal hour, at about the same time as the legs. These findings were confirmed by Auerbach (1936). The haltere bud can be recognized by the end of the larval period, at 4-4½ days (Chen). It is completely formed during the first 5 hours of the prepupal period and is segmented at the end of that time. Robertson (1936) gives a figure of a 2-hour prepupa in which the haltere has two segments. The haltere bud evaginates a little later than the wing bud (Robertson). The leg buds appear in 32-hour larvae (Chen) and begin to segment during the second day (Balkashina). Auerbach reports that segmentation begins during the third day. The leg buds are everted at about the same time as the wing buds, in the 5-hour prepupal stage (Robertson). The antennal bud can be recognized as a separate part of the cephalic complex after 48 hours of larval development (Chen); it begins to segment during the fourth day (Balkashina) and segments rapidly during the first few hours of the prepupal stage.

Robertson determined that the imaginal hypodermis is formed by the fusion of the lateral borders of the dorsal mesothoracic discs. This fusion occurs first ventrally, then laterally, and finally along the middorsal line. In a 5-hour prepupa the thoracic hypodermis is completely renewed ventrally and laterally, but a strip of larval hypodermis still persists on the dorsal surface. In a

7-hour prepupa the imaginal hypodermis has completed its development and has fused dorsally. In tetraltera type IVe the development of the thoracic hypodermis clearly was halted at the 5-hour prepupal stage; the imaginal hypodermis has grown ventrally and laterally, but has not completely replaced the strip of larval hypodermis in the middorsal line. This indicates that only imaginal and not larval hypodermis can grow bristles and microchaetes. Growth of the thoracic hypodermis and differentiation of the wing occur simultaneously in the first 5-6 hours of the prepupa, and these two processes are most affected by tetraltera. Growth and segmentation of the haltere bud also occur in this period. The simultaneous occurrence of these processes may prove important in understanding how the tetraltera locus acts in altering the developmental processes that produce the tetraltera phenotypes.

Because tetraltera produces such large changes in the phenotype, it probably affects processes early in development. To determine just how early the difference in development between normal and tetraltera could be detected, accurately timed larvae were raised and dissected and the dorsal mesothoracic discs observed. Since only about 15 per cent of the flies in any one culture show the character, it was thought unprofitable to fix and section the larvae to examine the discs.

First, mature larvae within 1 hour of pupation were dissected. The live larvae were dissected in Ringer's solution. No fixative was used because this might affect the size of the discs. Distinct differences in the size and differentiation of the dorsal mesothoracic discs were visible at this stage. Figure 5d shows the right and left dorsal mesothoracic discs from a tetraltera larva. The left disc, when compared to the figures given in Chen and Auerbach, proved to be quite normal, but the right one is much smaller and much less differentiated. There is no trace of the wing bud (the horseshoe-shaped structure in the center of the left disc) in this disc; only the outer circular disc has formed. Many other variations in the wing disc seen at this stage; the disc figured is one of the more extreme variations from wild type development. A census of the larvae at this stage revealed that 66 had both wing discs normal, 17 had one wing disc smaller and altered and the other normal, and 4 had both wing discs smaller and less differentiated than normal. This gives a penetrance of 24.1 per cent for the character in the mature larval stage, which is well within the range of variation of the adult penetrance.

A second series of dissections was made with larvae 3 days (72 hours) old. Dissections of the discs at this age are more difficult, but again some distinct differences were seen. Most of the larvae had normal dorsal mesothoracic discs, as before, but about 15 per cent showed differences in size and amount of differentiation between right and left discs (fig. 5e). At this stage the normal disc has a single circular ridge, and one of the variations observed was the absence or partial absence of this ridge. There is some variation in size in the normal discs in different flies at this stage, and no clear case was found where both discs were definitely smaller than normal. Differences could be, however, perceived between the right and left discs in the same fly.

Dissections of earlier larvae (55 hours) were attempted but were found to be

impracticable. Auerbach also learned that larvae so young could not be dissected successfully. The discs are very small, hard to locate, and difficult to identify when dissected out.

The action of the tetraltera locus occurs, or at least begins, therefore, at some unknown time previous to 72 hours of larval life.

In another approach to this problem a large number of adult tetraltera flies were examined and a table made of correlations between wing types and changes in the thorax, scutellum, and bristles (table 7). In addition, a large number of tetraltera wing types Ia, b, c, and d were examined, but since they all had completely normal thoraxes they were not recorded. In the condensed table of correlations of wing and thorax changes (table 6) a general correlation

TABLE 6  
CORRELATIONS OF WING AND THORAX CHANGES IN TETRALTERA PHENOTYPES

Wing type <sup>a</sup>	Thorax types					
	IVa	IVb	IVc	IVd	IVe	IVf
IIa . . . . .	1	1	1	1	0	0
IIb . . . . .	2	2	0	0	0	0
IIc . . . . .	2	4	8	10	0	0
IId . . . . .	4	8	14	13	0	0
IIIa . . . . .	2	3	40	34	8	3
IIIb . . . . .	0	0	11	5	11	5

<sup>a</sup> Wing types are arranged in series from least extreme to most extreme, IIa to IIIb; thorax types are arranged in series from least extreme to most extreme, IVa to IVf.

is seen between the amount of reduction in the wing and in the thorax. Hemithorax flies (IVf), exemplifying the most extreme change in the thorax, occur only with wing types IIIa and IIIb, the most extreme types of wing reduction. Rarely a hemithorax fly may have a IIc wing (fig. 3f); usually only IIIa or IIIb wings occur with this type of thorax. IIIb wings (wings absent) never occur with a normal thorax, but only with a IVc, d, e, or f thorax—a thorax in which at least the scutellum is absent. On the other hand, type I flies always have completely normal thoraxes, and type IIa and IIb flies have only small changes in the thorax—types IVa, b, c, or d.

The “doubled” thorax, type IVa, apparently occurs at random with any of the class II or class III types, except perhaps IIIb.

Assuming that those structures which are altered only when some other structure has been altered also are differentiated later than that structure, then certain conclusions may be drawn about the relative time of differentiation of parts of the dorsal mesothoracic disc. First, the wing is probably the last part of the mesothoracic disc to be differentiated, since changes in it may occur without simultaneous changes in the thorax, whereas changes in the thorax are always accompanied by large (class II or III) changes in the wing. The scutellum, on this basis, is the last part of the thorax to be differentiated, since it may be absent without any other change occurring in the thorax and

TABLE 7  
CORRELATION OF WING AND THORAX EFFECTS OF THE TETRALTERA GENOTYPE

Wing	Thorax	Bristles*								Remarks
		sc a	dc a	ps a	sa a	npl a	ps	hu a	p	
IIc	IVa									
IId	IVa									
IIIa	IVc IVd	—	—	—	2					whorl of microchaetes
IIIa	IVa			2	—					
IIIa	IVa									clear double thorax
IIc	IVb IVd			3	sm	sm				microchaete whorl
IIIa	IVc	—	—	1	sm	sm				
IIIa	IVc IVd	—	—	sm						microchaete whorl
IId	IVa			—	sm					

\* Each horizontal line represents a single fly; the upper half of the block represents its left side, the lower half, the right side. Abbreviations are as follows:

—	.....	bristle absent	sm	.....	bristle smaller than normal
1	.....	one extra bristle present	der	.....	bristle deranged
2	.....	bristle doubled	abn	.....	bristle abnormal
3	.....	two extra bristles present	msp	.....	bristle out of place

TABLE 7—(Continued)

Wing	Thorax	Bristles*											Remarks	
		sc a	sc p	do a	do p	pa a	pa p	sa a	sa p	npl a	npl p	ps		hu a
IIc	IVc IVd	—	—	—	—	—	—	—	—	—	—			whorl microchaetes
IId	IVb IVd					—	—	—	—					
IId	IVc IVd	—	—			—		—						whorl microchaete
IIa	IVc IVd	—	—			—		—	sm	sm				whorl of microchaetes
IIIa IIIa	IVc IVd IVc IVd	— —	— —	der der	der der	— —	— —							whorl on both sides
IId IIb	IVc IVa	— —	— —		2	—		sm						
IIc IIId	IVc IVd IVc IVd	— —	— —			— —	— —		—					whorl of microchaetes
IIIa	IVc	—	—			abn		2						whorl of microchaetes
IIIa	IVc	—	—			abn		1						whorl of microchaetes
IIIa	IVc	—	—			—		sm	sm					



TABLE 7—(Continued)

Wing	Thorax	Bristles <sup>a</sup>								Remarks
		sc a	dc a	pa a	sa a	apl a	ps	hu a		
IIId	IVb			3					whorl of microchaetes	
IIIf	IVc IVd	— —			2				whorl	
IIId	IVc IVd	— —	— —	— —	sm				slight whorl	
IIId	IVc IVd	— —	— —	— —					right haltere gone, whorl	
IIc	IVb			abn	sm				whorl	
IIc	IVa								very clear double thorax	
IIId IIIf	IVc IVd IVc IVd	— — — —	— — — —	— — — —	— — — —					
IIb	IVa									
IIIf	IVf									
Ic	IVc IVd	— —	— —	— —					whorl	

TABLE 7—(Continued)

Wing	Thorax	Bristles*								Remarks
		sc a	dc a	pa a	sa a	npl a	ps	hu a	p	
IIIa	IVc IVd IVe	—	—	—						
IIIa	IVc IVd IVe	—	—	—						
IIIa	IVf									
IIIa	IVf									halterc gone
IIc	IVc IVd	—	—	—	—					
IIIb	IVc IVe	—	—	—	—					
IIIb	IVc IVf		—	—	—	sm				
IIIb	IVc IVe	—	—	—	—					
IIIa	IVc IVe	—	—	—						whorl
IIIa	IVc IVe	—	—	—	—	sm				
IIIb	IVe		—	—	—	—		—	—	





TABLE 7—(Continued)

Wing	Thorax	Bristles <sup>a</sup>												Remarks				
		a	so	p	a	do	p	a	pa	p	a	sa	p		a	npl	p	a
IIId	IVc IVd	—	—	—				msp	msp	sm								whorl
IIIIa	IVc IVd	—	—	—				—	—	—								slight whorl
IIIIa	IVc IVd	—	—	—	—	—	—	—	—	sm	sm							whorl
IIIIa	IVc IVd	—	—	—	—	—	—	—	—	—	—							whorl
IIIIa	IVc IVd	—	—	—				—	—	—	—							whorl
IIIIa	IVc IVd	—	—	—				msp	msp	—	—							whorl
IIIIa	IVc IVd	—	—	—				—	—									whorl
IIIIa	IVb																	postalar region abn.
IIIIa	IVc IVd	—	—	—	2	—	—	—	—									
IIIIa	IVc IVd	—	—	—	—	—	—	—	—	—	—							whorl

TABLE 7—(Continued)

Wing	Thorax	Bristles*												Remarks
		sc a	dc a	pa a	sa a	npl a	ps	hu a						
IIIa	IVb												slight whorl	
IIId IIIb	IVb IVc IVe	— —	— —	abn —	— —									
IIIa				abn										
IIId	IVc IVd	— —		—										
IIIb	IVe		— —	— —	— —									
IIIa	IVc IVd	— —	— —	— —	— —								whorl	
IIIb	IVc IVd	— —	— —	— —	— —								whorl	
IIIb	IVc IVd	— —	— —	— —	— —									
IIIa	IVe		— —	— —	— —									
IIIb	IVc IVf	— —	— —	— —	— —									

TABLE 7—(Continued)

Wing	Thorax	Bristles*												Remarks	
		a	sc	a	dc	a	ps	a	sa	a	npl	ps	a		hu
IIIa	IVe			—	—	—	—	—	—	—					
IIIa	IVc IVd	—	—			—			—						
IIId IIIa	IVc IVd IVc IVd	— —	— —	—	—	—	abn	sm sm	sm sm						whorl
IIIa	IVc IVd	—	—	—	—	—	—	—	—						
IIIa	IVc IVd	—		—	—	—			—						whorl
IIc	IVb IVd						abn								whorl
IIIa	IVc, d	—	—			—	—	—	—						
IIIa	IVc, d	—	—						—						
IIIb	IVc, d	—	—			—	—	—	—						
IIIa	IVb, d							sm	sm						

TABLE 7—(Continued)

Wing	Thorax	Bristles*								Remarks
		a so p	a do p	a pa p	a sa p	a npl p	ps	hu a p		
IIIIa	IVc, d	—	—	—	—	—				
IIIIa	IVc, d	—	—	—	—	—			whorl	
IIIIa	IVc, d	—	sm	—	—	—				
IIIIc	IVc, d	—	2	—	—	—			whorl	
IIIIc	IVc, d	—	—	—	—	—				
IIId	IVc, d	—	—	—	—	—				
IIIIa	IVc, d	—	—	—	—	—				
IIIIa	IVc, d	—	—	—	—	—			whorl	
IIIfb	IVb IVd			ex.	sm					
IIIfb	IVb	2								





its bristle pattern; but in all I Ve flies, where the imaginal hypodermis does not meet in the midline, the scutellum is absent. The formation of the normal bristle pattern is probably dependent upon the meeting of the imaginal discs in the dorsal midline, because when the discs do not meet the bristle pattern is abnormal and many of the bristles are absent. The production, by the tetraltera genotype, of such large variations in the phenotype probably means that the times of determination of these various parts of the dorsal mesothoracic disc, wing scutellum, and mesonotum are close together—so that slight differences in the time of the beginning of the action of the tetraltera genotype can produce these wide variations in the resulting phenotype.

Since the tetraltera factor can affect the determination of the wing, the scutellum, and the whole dorsal mesothorax, it probably produces its effect before these various parts are determined and separated in the disc. The stage in the development of the normal fly in which these parts are determined is as yet unknown. The first visible differentiation in the dorsal mesothoracic disc in the larva occurs at about 60 hours after hatching, when the first cross ridge appears on it (Auerbach). The fate of this cross ridge is unknown. Since distinct differences in size and differentiation can be seen between right and left discs in tetraltera 72 hours after the larvae hatch from the eggs, the action of the tetraltera locus must occur some few hours before this, probably earlier than 60 hours, before any visible differentiation of the disc has occurred.

No segmentation was seen in the abnormal larval wing discs dissected from mature larvae. The segmentation of the wing anlage that take place in some tetraltera types probably occurs in the first 5 hours of prepupal development, when the haltere disc is segmented. Segmentation of the haltere and the differentiation of the wing occur simultaneously in the first 5 prepupal hours; therefore, the tetraltera mutant, unlike what has been assumed for aristapedia, does not produce its effect by shifting the time of differentiation of the disc. Instead, the tetraltera mutant must somehow—assuming that wing and haltere discs may be determined at different times—affect the time or the type of determination of the dorsal mesothoracic disc early in larval development so that it develops as a haltere instead of a wing during this early prepupal period.

Segmentation in the tetraltera wing may be a secondary phenomenon dependent upon the amount of growth or the growth gradient of the distal end of the dorsal mesothoracic appendage. The primary effect of the tetraltera factor occurs early in development and may affect the determination of the disc so that the tip of the wing bud is reduced in size. Then, later in development, during the first 5 prepupal hours, when the haltere and wing discs are growing differentially, the amount of growth of the distal end of the appendage determines the absence or presence of segmentation. If the tip is growing as in a normal wing, any segmentation is masked by the great growth of the tip into the wing plates. If, however, the tip has been reduced in size by the previous action of the tetraltera factor, it will not grow so rapidly and any tendency towards segmentation can proceed and produce the type of segmentation of the normal haltere. In the morphological study the IIa types, in which the tip of the wing appendage is largest, showed little or no segmentation, whereas the

IIc or IId types, in which the tip of the wing appendage is greatly reduced, showed clear segmentation, indicating that the amount of segmentation depends upon the size of the wing and its bud.

In aristapedia, apparently no change in the size of the antennal disc is produced by the aristapedia mutant; the 2-day aristapedia antennal disc is the same size as the normal 2-day antennal disc. The effect of the aristapedia locus is to change the determination of the 2-day antennal disc so that it can respond to the leg segmentation evocator. Both Braun (1939b, 1940) and Waddington (1939) showed that these antennal tarsi are affected by the leg mutants "dachs," "approximated," and "four-jointed," but not by the antennal mutants "thread" and "aristaleess." The developmental system of the aristapedia antenna is therefore completely that of a leg and not of an antenna. Waddington describes two other alleles of aristapedia, (1) aristapedia-Bridges, in which the change from antenna to leg is less marked, only the basal part of the arista being changed in a tarsal direction, whereas the third antennal segment and the distal part of the arista remain fairly normal; and (2) aristapedia-Spencer, which is a slightly stronger allele of aristapedia. Each stock varies markedly; by making various compounds of these alleles a whole series of forms may be obtained in which different proportions of the arista are affected. These aristae are not intermediates between legs and aristae; certain parts are strictly leg-like, whereas the other parts are strictly aristalike, the transition zone between the parts being very narrow. The transition from arista to tarsus occurs from proximal to distal; that is, the proximal end of the arista is the first part to become tarsuslike, and the distal tip is the last. These forms seem to show that no intermediate exists between arista and tarsus development; the disc must develop one way or the other. Waddington suggested some modification of the type of explanation found in Goldschmidt's theory of intersexuality as Goldschmidt had done before (1938): namely, that the phenomena are dependent on the time relations between the determination process and some other process which decides whether the determination shall be that of an arista or a leg (Goldschmidt's evocator). The transition from arista to tarsus occurs first proximally and may extend only a short distance beyond which the appendage is distinctly an arista. This fact accords with Goldschmidt's time-relation theory outlined previously (page 147). Here the time relations between the action of the aristapedia locus in affecting the ability or potency of the antennal disc to react to the leg evocator, and the diffusion of the leg evocator into the disc, are not completely synchronized; consequently, only the proximal part of the antennal disc can be affected by the evocator which is diffusing in while the rest of the disc goes on to form a normal arista.

The aristapedia locus has another phenotypic effect besides changing the arista to a tarsus. This is the production of irregular segmentation in the tarsus itself, affecting primarily the proximal region of the tarsus. Thus, the aristapedia locus is also concerned in the processes which determine the segmentation of the tarsus.

In each of the homoeotic mutants investigated, the homoeotic locus may do more than simply change one appendage into another: it may affect the de-

velopment of the whole disc from which the appendage is derived. In proboscipedia, in addition to changing the oral lobes into a labiumlike organ which may resemble a pair of antennae or tarsi, the homoeotic locus may change the labrum, maxillae, maxillary palpi, and all other mouth parts to resemble the biting type of lower insects, indicating that the entire labial disc has been affected. In bithorax and tetraptera, in addition to the change of the haltere to a wing, the metathorax may be enlarged and may bear bristles resembling those of the mesothorax. In tetraltera, in addition to the change of the wing into a haltere, the mesothorax may be reduced and the scutellum and bristles removed so that it resembles a metathorax. The condition in figure 3f is almost a perfect metathoracic mesothorax: the appendage is a haltere and the mesonotum resembles a metanotum; it is greatly reduced and bears no scutellum bristles.

Goldschmidt (1940) suggested that all these imaginal discs have identical potencies in the early larva or egg and that normally they become ready to receive induction at the right time, so that they are determined by the right inductive stimulus to form the normal appendage. These homoeotic mutants change the time when the discs become ready to receive induction, so that the discs respond to some abnormal induction and produce the abnormal appendage. A slight extension of this theory would account for the multiple changes found in the homoeotic mutants. Thus, in tetraltera if only the wing becomes haltere-like while the rest of the thorax develops normally, the wing-disc part of the dorsal mesothoracic disc is affected according to the above system and determined to develop into a haltere instead of a wing. If, however, the tetraltera locus acts earlier in development or perhaps at a greater rate, the whole dorsal mesothoracic disc can be influenced abnormally and determined to develop into a metathorax. The stages in the reduction of the mesothorax, types IVc, IVd, IVe, and IVf, may be regarded as steps in the change of a mesothorax into a metathorax in each of which more of the mesothorax becomes metathorax-like, just as more of the arista became leglike in the series reported by Waddington. These changes, again, result from slight differences in the times of induction, by the evocator, of the whole dorsal mesothoracic disc. A similar explanation would hold for the variations in the metathorax observed in tetraptera and bithorax, and for those in the mouth parts other than the oral lobes observed in proboscipedia.

## DISCUSSION

The present work on tetraltera has evinced a number of facts which add to the discussion of the problem of homoeosis presented by Goldschmidt (1940). The morphological study of tetraltera showed the complete correspondence of the series of intermediates between wing and haltere found among the tetraltera phenotypes with the series of intermediates in tetraptera, which further demonstrates the homology of wing and haltere, and shows that the same or similar embryological processes are affected by these two mutants. The taxonomic parallel to the tetraltera mutant, pointed out by Goldschmidt (1940) to exist in the aberrant termitophile fly *Termitoxenia* and its allies, was studied

in Kemner's (1940) review of the *Termitoxenia* group. It was found that the tetraltera phenotype which is called IIa in this study—a type intermediate between wing and haltere which resembles neither very closely—resembles the *Termitoxenia* wing almost exactly. Goldschmidt also pointed out that in many species of Hymenoptera, Neuroptera, Strepsiptera, and Homoptera the meta-thoracic wings may be reduced to halterelike rudiments, and that in parasitic Hymenoptera, Orthoptera, and Homoptera the mesothoracic wings may be halterelike, emphasizing the evolutionary importance of the existence of these homoeotic mutants in providing a model for the origin of these types.

Stern (1940), studying a number of hemithorax flies produced by chance in vestigial and other stocks, learned that in these flies the sternopleural region develops normally even if the dorsal mesothoracic discs fail to evert. However, if the mesothoracic leg is missing, as it sometimes is in purple and other stocks, the sternopleural region is always missing or abnormal. Stern interpreted this to indicate that the sternopleural region is not sometimes formed by the dorsal mesothoracic disc, as Sturtevant (1929B) and Noujdin (1936) had thought, but is formed invariably by the ventral mesothoracic disc. I examined more than 150 hemithorax flies from the tetraltera stock; all had normal sternopleural regions. This confirms Stern's conclusion that the hypodermis which forms the sternopleural region must come from the ventral, and not the dorsal, mesothoracic disc.

The morphological study of tetraltera also disclosed unusual flies in which the wing, the scutellum, or the whole mesonotum were apparently doubled. Stern (1940) noticed several examples of doubled sternopleural region, in flies lacking mesothoracic legs, and suggested that this splitting or doubling of parts of the mesothorax is caused by the great upsets in the ontogeny of the parts. A similar explanation is indicated for the doubling occurring among tetraltera phenotypes.

The genetic analysis of tetraltera revealed that a normally recessive mutant was located in the right arm of the third chromosome at or near locus 48.5. Tetraltera, as well as most of the other homoeotic mutants, shows an incomplete penetrance of the character; that is, only a certain percentage of the flies genetically homozygous for the mutant show the phenotype. The penetrance of the character in the offspring remains the same whether phenotypically normal or phenotypically tetraltera flies from the tetraltera stock are used in breeding.

When certain other mutants were crossed to tetraltera, some noteworthy interactions took place. Two large inversions in the second chromosome, *Curly* and *Glazed*, were found to inhibit the production of the tetraltera phenotype; *Glazed* inhibits tetraltera completely, *Curly* to a less degree. Several mutants were found which enhanced the tetraltera factor and acted as dominigenes to it, enabling a fly heterozygous for tetraltera to appear phenotypically tetraltera. Among the mutants producing this effect are the large third chromosome inversion, *Moiré*, the fourth chromosome mutants, *eyeless* and *eyeless-Dominant*, and the third chromosome inversion *Dichaete*. In addition to its dominigene effect, the mutant *eyeless-Dominant* which is really a small duplication acts to increase the penetrance of tetraltera when

the tetraltera factor is homozygous. This dominigene action of eyeless and eyeless-Dominant is unusual; they are not known to act on other mutants. The dominigene action of Moiré is not surprising, however, since it shows the same action with the second chromosome mutant dumpy and the third chromosome mutant dumpoidy (unpublished data), both of which affect wing development. Thus Moiré probably acts somehow during the determination and differentiation of the wing to lower the threshold of some reaction, so that a single dose of the mutant locus—dumpy, dumpoidy, or tetraltera—can produce the same effect in the resulting phenotype as is normally produced by a double dose of the mutant locus.

The penetrance of homozygous tetraltera has been shown to have an inverse relationship with temperature: less than 1 per cent at 29° C., about 8 per cent at 25° C., increasing to about 15 per cent at 20° C., and about 36 per cent at 14.4° C. Astauroff (1930) reported a diametric relationship for tetraptera, in which the penetrance varied directly with the temperature: from 0–1 per cent at 17° C. to about 35 per cent at 25° C. Since the embryological processes resulting in the tetraptera phenotype, the changing of a haltere anlage to produce a wing, are exactly opposite from the processes resulting in the tetraltera phenotype, the changing of a wing anlage to produce a haltere, this opposite relationship between penetrance and temperature existing in the two stocks probably has some bearing on the action of the processes producing opposite results, halteres and wings. The action of temperature on development seems to be that of a differential acceleration or retardation of certain processes in relation to other processes of development. We may assume, therefore, that the production of a wing instead of a haltere requires the differential acceleration of some reaction or reactions which is increased by higher temperature occurring during the determination and differentiation of the wing and haltere discs, whereas the production of a haltere instead of a wing requires the differential retardation of these same or similar processes which is increased by lowered temperature.

The experiments on the temperature-effective period showed that no stage in the development of tetraltera exists in which a heat or cold shock will affect materially the penetrance of the character in the adult. The increase in penetrance produced by prolonged cold treatment must therefore result not from the cold itself but from its prolongation of development. This must mean that the reaction or reactions which lead to the production of the tetraltera phenotype are not themselves affected by changes in the temperature but rather that some threshold phenomenon obtains. Prolongation of early development permits the altered reaction system present in genetically tetraltera flies to function longer. Thus the system reaches the threshold necessary to produce the tetraltera phenotype more often than in normal development. Under normal conditions development may only be delayed, and the tetraltera phenotype produced by chance combinations of incidental environmental conditions.

Changes in the food conditions of the developing larvae were also found to affect slightly the penetrance of tetraltera. The addition of Nipagin to the normal laboratory food prolongs the development of the larva by checking

the growth of the yeast in the culture bottles and thus decreasing the amount available as food. The Nipagin treatment resulted in a 2-day prolongation of development and an increase of 3.4 per cent in the penetrance, which is quantitatively about the expected increase, as a 3-day prolongation produced by an 8-day cold treatment raised the penetrance 5.5 per cent. Again, not the Nipagin itself but the prolongation of development, caused by the addition of Nipagin to the food, increased the penetrance, probably in the same manner as with cold treatment.

Development takes about a day longer in phenotypically normal tetraltera flies than in wild-type flies, 2-3 days longer in phenotypically tetraltera flies. Some difference exists in the total physiology of development in the two sexes which causes the total time of development to be longer in the male than in the female. This probably explains the higher penetrance of tetraltera in males, for any prolongation of development, no matter how produced, acting at the proper stage in early larval development, increases the penetrance of tetraltera.

Goldschmidt (1940) pointed out that all the homoeotic mutants vary greatly in their expression. This is especially true of tetraltera. He suggested that the genetic effect of the mutant locus affects the developmental processes in question only within a small range of time, so small that the normal fluctuation of developmental speed caused by external and internal environment may easily shift the decisive event below the threshold. The results of my study are explained very well by this hypothesis. The action of the various environmental factors which prolong early development and thus increase the penetrance of tetraltera must be to prolong the action of the tetraltera locus, so that in a greater percentage of flies this threshold is reached and the tetraltera phenotype is produced. The action of the genetic suppressers and enhancers of tetraltera might also be to shorten or prolong this early critical period.

Production of the tetraltera phenotype may be summarized as follows. The tetraltera locus shifts the time of differentiation of the dorsal mesothoracic disc so that it coincides with the inductive action on the dorsal metathoracic disc. Slight variations in the rate of development, caused by the external and internal environment, may shift this time so that much, little, or none of the disc will be affected, resulting in adults in which much, little, or none of the thorax and wing region is affected, or in which the region is affected to a greater or less degree, producing a wide variety of phenotypes. If the disc is affected, the tip of the wing bud may be reduced in size or its growth gradient may be altered. This, in turn, later in development, when the haltere and wing discs are growing differentially, may determine the presence or absence of segmentation: if little affected, the tip will grow enough to mask any tendency toward segmentation; if much affected, the tip will not grow so rapidly and any tendency toward halterelike segmentation can proceed.

## CONCLUSION

A discussion of homoeotic mutants and their importance in evolutionary considerations was given by Goldschmidt (1940). These homoeotic mutants are good examples of large mutations, single mutants acting early in development and producing large changes in the external appearance of the animal, changes so large as to be characteristic of a different genus, family, or order. The changes in *Drosophila* caused by the mutant tetraltera produce a fly resembling, with respect to the wings, the aberrant dipteran *Termitoxenia*, a member of an altogether different family. The mutant proboscipedia produces mouth parts whose structure is radically different from that of normal *Drosophila* and which resembles instead the biting mouth parts characteristic of the lower orders of insects. No member of the order Diptera has mouth parts resembling even remotely those of proboscipedia flies. The mutants bithorax and tetraptera also affect characters of high taxonomic value. The presence of one pair of wings instead of two is a character distinguishing Diptera from other orders of insects, but these mutants produce two pairs of wings, resulting in a "fly" which no longer belongs to the order Diptera.

Genetically, the homoeotic mutants in *D. melanogaster* are of unusual interest because, with the exception of the homoeotic Lobe allele mentioned by Goldschmidt (1940), they all lie in the same region of the right arm of the third chromosome. When Balkashina (1929) localized aristapedia in the same region with bithorax, bithorax-b, and tetraptera, she mentioned this as being a remarkable coincidence. Since that time several other homoeotic mutants have arisen and all have been found to lie in this same region. Goldschmidt (1938) mentioned this in support of his hypothesis that no "genes" exist, but that the chromosomes themselves act as units in heredity, controlling the normal development of the animal. Thus, on this basis there is not an accidental cluster of homoeotic genes in this region; rather, this whole region of the right arm of the third chromosome acts as a whole in normal development in regulating the determination and development of the imaginal discs. Various upsets in the pattern of this part of the chromosome produce the different homoeotic phenotypes. So far, no additional evidence supports or disproves this explanation. No visible aberrations in the structure of salivary gland chromosomes of the homoeotic mutants have yet been seen. An investigation of this whole third right arm region, using the "lampbrush" chromosome technique described by Kodani (1941) may disclose some special relationship of these loci to the b-heterochromatic regions of the chromosome.

Studies of the various homoeotic mutants have brought to light many facts important in embryology and comparative anatomy. The work on bithorax, tetraptera, and tetraltera demonstrated the homology of wing and haltere and of mesonotum and metanotum. Previously, the halteres of Diptera were supposed to represent the second pair of wings of other insect orders; these mutants prove this homology clearly. The study of aristapedia revealed the homology of the antenna and tarsus, proving that the antenna is a homologue of the mouth part leg series of segmental organs, rather than a separate pre-



oral structure outside this series, as is suggested by some comparative anatomists. The eye mutants, Lobe and kidney, may indicate that the eye, too, is a member of the same series, and homologous to the other members. However, these eye mutants may not be strictly comparable to the other, clearly homoeotic, changes. Both the eyes and the antennae come from a single disc, and these substitutions of an antenna for an eye may represent, simply, an upset in the development of the original disc prior to the determination of the eye and antennal parts. The mutant proboscipedia shows that the oral lobes of Diptera are homologous to the labium of other insects and are not the derivatives of maxillae, as was supposed by Lowne (1890). The basal part of the proboscipedia organ may represent a modification of the glossae and paraglossae of lower insects, whereas the lateral parts are comparable to the labial palpi of lower insects, no traces of which palpi are normally present in the oral lobes of flies. The presence of tarsuslike and antennalike structures on these modified oral lobes is another indication of the homology of these mouth parts with the antenna-mandible-maxilla-labrum-walking leg series.

The development of homoeotic types seems best explained by the system proposed by Goldschmidt (1938, 1940), already elaborated here; namely, all imaginal discs have identical potencies in early development, before they become determined by the proper inductive stimulus. Each homoeotic mutant acts to shift the time of the determination of the disc in question to that of some other disc, causing the homoeotic development. This would explain the appearance in proboscipedia flies of either tarsuslike or arisalike structures, depending upon whether the imaginal discs producing the mouth parts (labial buds) have had their time of determination shifted to coincide with that of the leg, or that of the antenna. Phenocopies of certain of these homoeotic mutants have been produced: Enzmann and Haskins (1939) produced types similar to the homoeotic kidney and tetraltera mutants by neutron bombardment; Rapoport (1939) produced aristapedia types by treating larvae with certain chemicals. Thus the time of determination of the imaginal discs may be shifted by external agencies as well as by an inherited mutation.

Goldschmidt (1940) pointed out the importance of the facts derived from studies of these homoeotic mutants to a general analysis of evolution in terms of his concept of systemic mutations and macroevolution. If an embryological system of the type described underlies the determination and differentiation of the appendages, and if this system is controlled by the genotype in the manner described in the theory of balanced reaction velocities in time, then a system obtains in which small changes in the genotype controlling either the speed of differentiation, the gradients of segmentation, or the time of induction of the different discs, may lead to sudden macroevolutionary steps in any and all details of segmental divergence. In the course of phylogeny, in other words, one major and a few minor changes in genotype can suddenly produce dorsal appendages and then change them into wings or halteres, thus demonstrating that macroevolution is possible without the accumulation of micromutations under the pressure of selection.

## SUMMARY

(1) The recessive mutant tetraltera, discovered by Dr. Goldschmidt in December, 1934, in a cross of Beaded  $\times$  Beaded, was localized in the right arm of the third chromosome, approximately at locus 48.5.

(2) The action of the mutant locus is suppressed by the inversions Glazed and Curly and is enhanced by the inversions Moiré and Dichaete and by the mutants eyeless and eyeless-Dominant.

(3) Several mutants occurred in the tetraltera stock during this investigation, including striped thorax (an allele of ebony, 3-70.7), glassy eyes (an allele of glass, 3-63.1), dumpoidy wings (in the right arm of the third chromosome somewhere near locus 85 to 90), curled wings (lost before it was localized), and plexus wings (an allele of plexus, 2-100.5).

(4) Tetraltera did not show allelism with any of the other third chromosome mutants tested: maroon, pink, blistery, bithorax, bithorax-Dominant, proboscipedia, aristapedia, and kidney.

(5) A cytological study of the salivary gland chromosomes revealed the presence of an inversion which was lethal when homozygous in the right end of the right arm of the third chromosome, resembling In3RC in its breakage points, a heterozygous deficiency for band 87E3, and a heterozygous translocation or transposition at band 98C1. Probably none of these aberrations are related directly to the production of the tetraltera phenotype, since they are all farther to the right than the locus of tetraltera.

(6) The phenotypic expression of tetraltera is extremely variable. All sorts of intermediates between a wing and a haltere may be produced; the mesothoracic appendage may be reduced further to a small one- or two-segmented palpus, or it may be absent. The scutellum and mesonotum and their bristles may also be altered or missing; in hemithorax flies the dorsal mesothoracic disc does not evert at all. In addition, the wing, the scutellum, or the mesonotum may be doubled. These phenotypic variations were divided into classes for convenience of study and description.

(7) Members of the tetraltera series of phenotypes were compared with those of the tetraptera series described by Astauroff, and corresponded exactly. Tetraltera was also compared with the aberrant dipteran *Termitoxenia*. The type which had been called IIa resembled the *Termitoxenia* wing almost exactly.

(8) The penetrance of the tetraltera factor at room temperature (20° C.) is about 15 per cent. At 29° C. it drops to less than 1 per cent, and at 14.4° C. it increases to 36.7 per cent.

(9) No sensitive period to cold or heat shocks could be found at any period in the development of tetraltera flies.

(10) The addition of 0.2 per cent Nipagin to the cornmeal-agar-molasses food prolonged development 2 days and increased the penetrance slightly. Partial starvation experiments (peptone) did not result in increased penetrance, probably because the prolongation of development occurred after the critical period for tetraltera.

(11) The total time of development of phenotypically normal tetraltera flies is about 1 day longer, and of phenotypically tetraltera flies 2-3 days longer, than that of wild flies.

(12) Larvae 72 and 96 hours old were dissected and at both ages differences between normal and tetraltera discs could be observed. The tetraltera discs were smaller and less differentiated than the normal discs.

(13) A table of correlations of the effects of tetraltera suggests that the wing is determined later than the scutellum, which in turn is determined later than the rest of the mesonotum. Each side of the mesothorax is completely independent of the other; one side will develop and differentiate normally even when the other disc does not evert.

(14) The possible action of the tetraltera locus in affecting the determination, differentiation, and segmentation of the dorsal mesothoracic disc and appendage and the evolutionary significance of this type of system are discussed.

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# EXPLANATION OF FIGURES 1, 2, 3

For these drawings, selected flies were preserved in 70 per cent alcohol, and mounted in glycerine on depression slides. Only the thorax is shown; normal wings, when present, are usually not shown.  $\times 100$ .

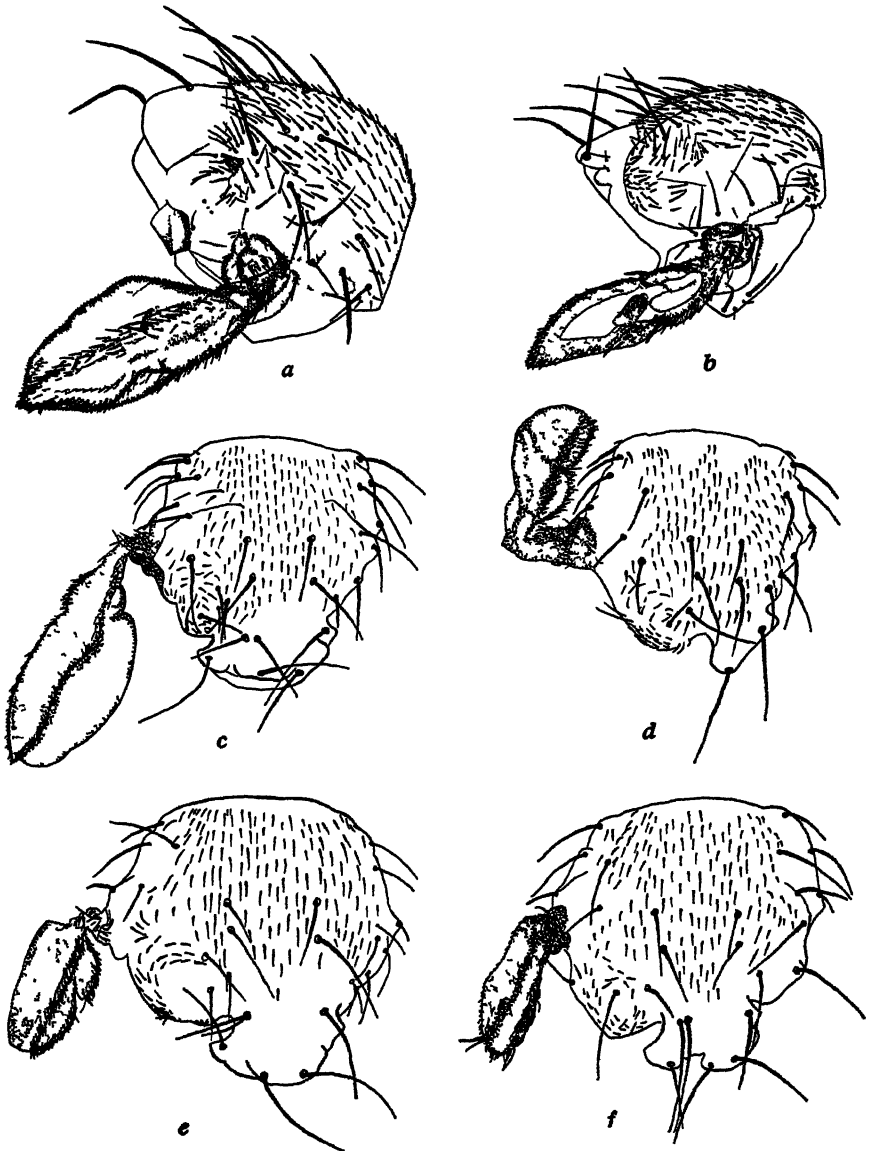


Fig. 1. (a) Right wing tetraltera IIa. (b) Right wing tetraltera IVb. Note lateral projection of scutellum and its small bristle (tetraltera IVb). (c) Left wing tetraltera IIa. Note extra bristles on scutellum (tetraltera IVb). (d) Left wing tetraltera IIb. Left half of scutellum missing (IVc). (e) Left wing tetraltera IIb. Extra bristles on scutellum (IVb). (f) Left wing tetraltera IIb. Note clawlike bristles on end of wing. Extra bristles on scutellum (IVb).

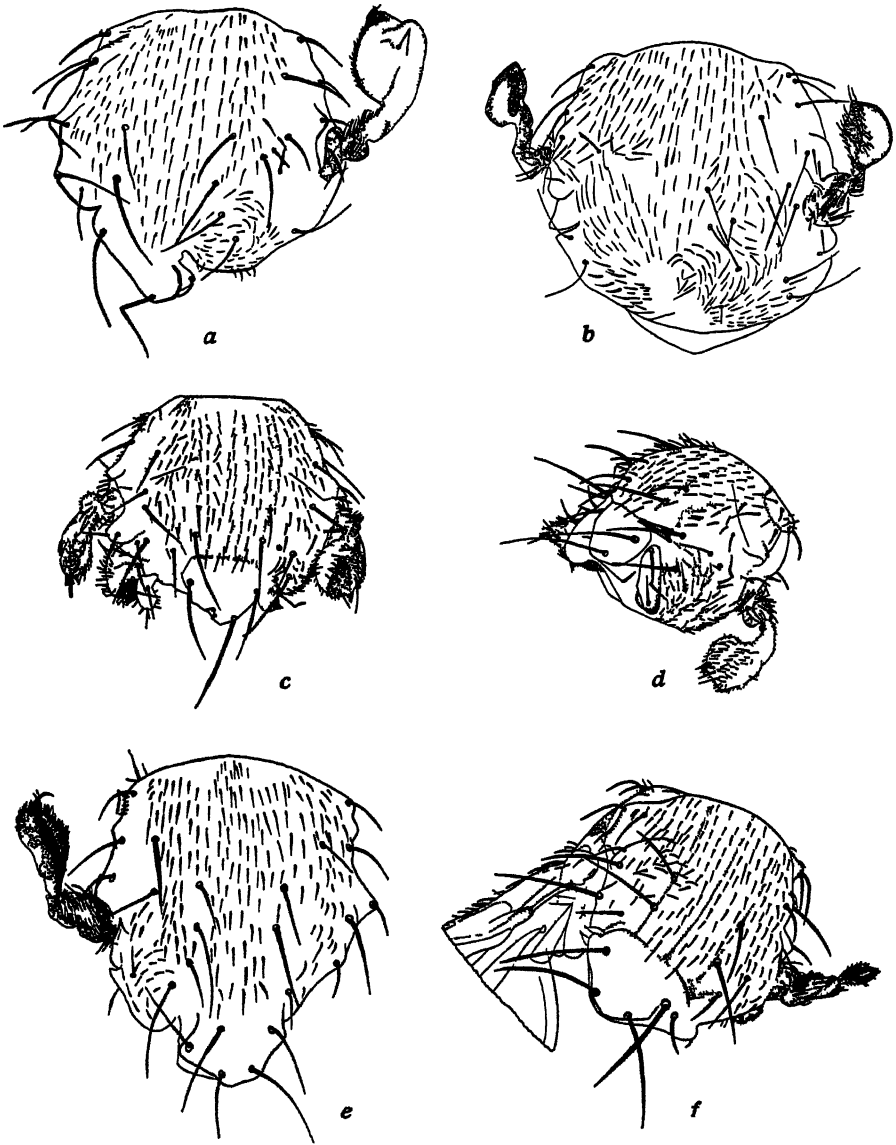


Fig 2. (a) Tetraltera IIb-IIc wing. Right half of scutellum missing (IVc). (b) Right wing tetraltera IIc. Both sides of scutellum missing (IVc). Note whorls of microchaetes. (c) Dorsal view of thorax of tetraltera fly, both wings IIc. Note outgrowth on left side (IVa). (d) View of same fly from left side, showing detail of left appendage. (e) Left wing tetraltera IIc-IIId. Note clawlike bristles on tip of "head," and extra bristles on scutellum (IVb). (f) Right wing tetraltera IID. Extra bristle lateral to right anterior scutellar bristle (IVb).

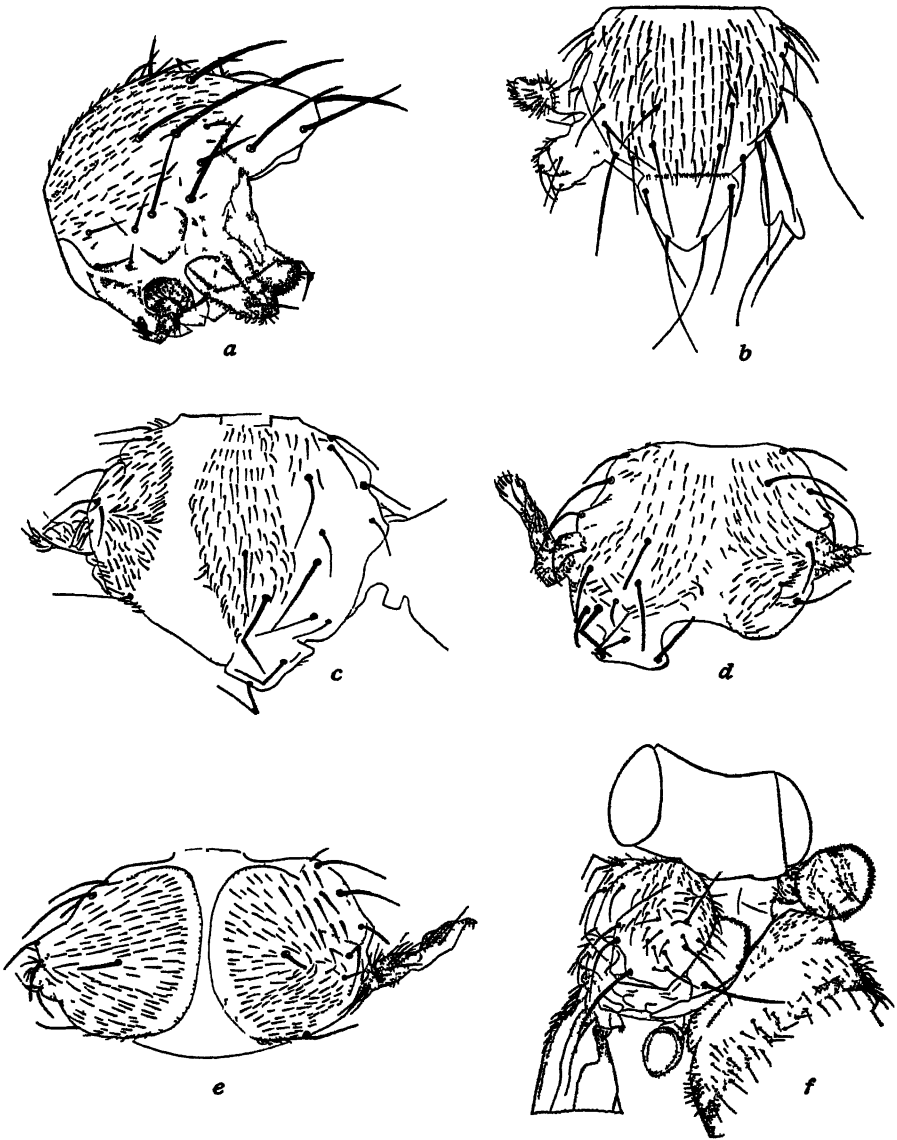


Fig. 3. (a) Left wing tetralter II d. Thorax tetralter IV a. (b) Left wing tetralter III a: two-segmented palpus. Large outgrowth posterior to wing, tetralter IV a. (c) Left wing tetralter III a: one-segmented palpus. Left half of thorax type IV e. (d) Right half of scutellum missing (IV c); extra bristle on left half of scutellum (IV b). (e) Tetralter IV e thorax. Dorsal mesothoracic discs fail to meet in dorsal midline. (f) Tetralter IV f, "hemithorax" fly. Right wing II c. Left side normal.



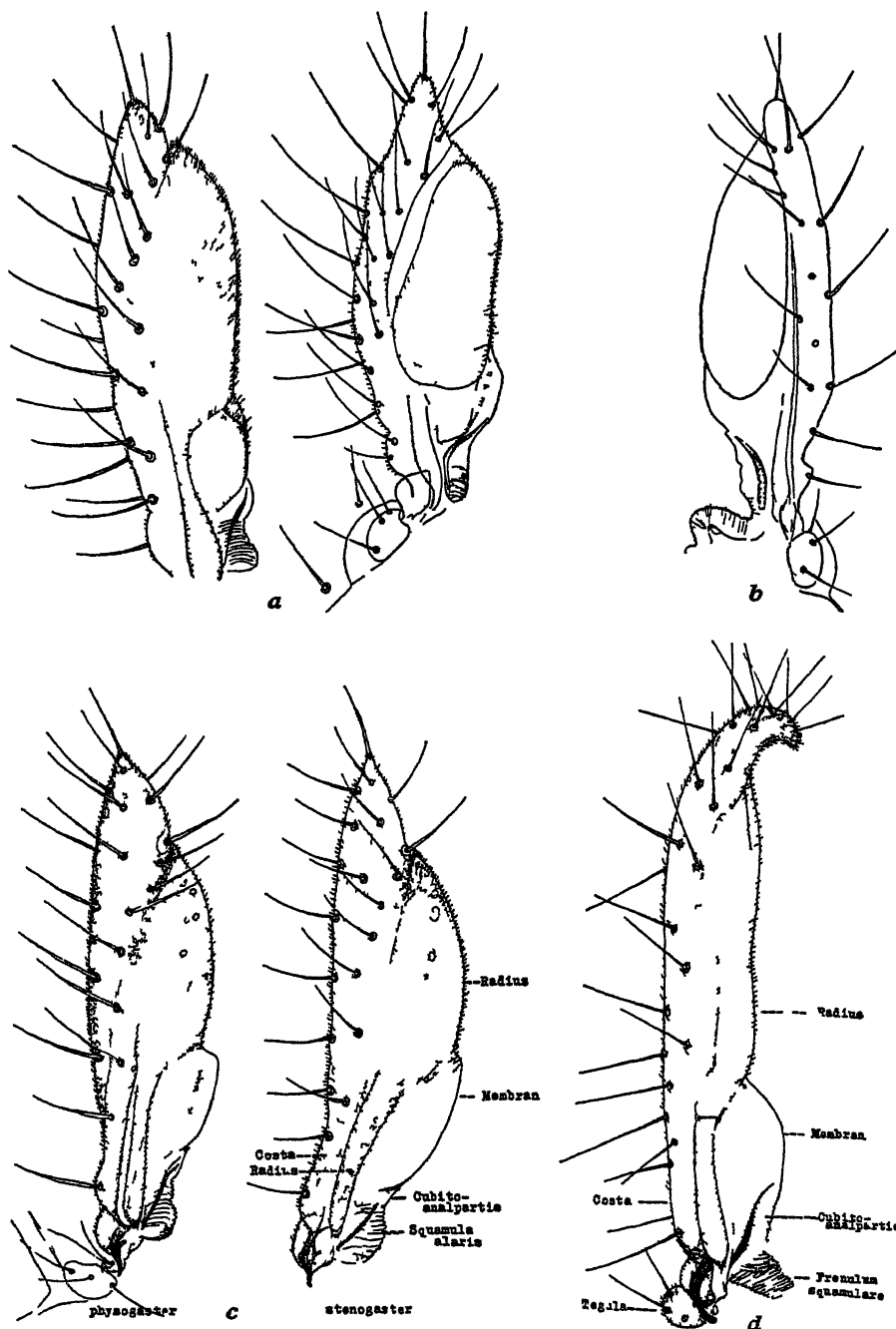


Fig 4 (a) Right wings of *Odontozenia breirostris* Schmitz, dorsal view (after Kemner)  
 (b) Right wing of *Termitozenia jagerskold* Wasmann, ventral view (after Kemner)  
 (c) Right wings of *Javanozenia punctiventris* Schmitz, dorsal view (after Kemner) (d)  
 Right wing of *Termitomyra mirabilis* Wasmann, dorsal view (after Kemner).

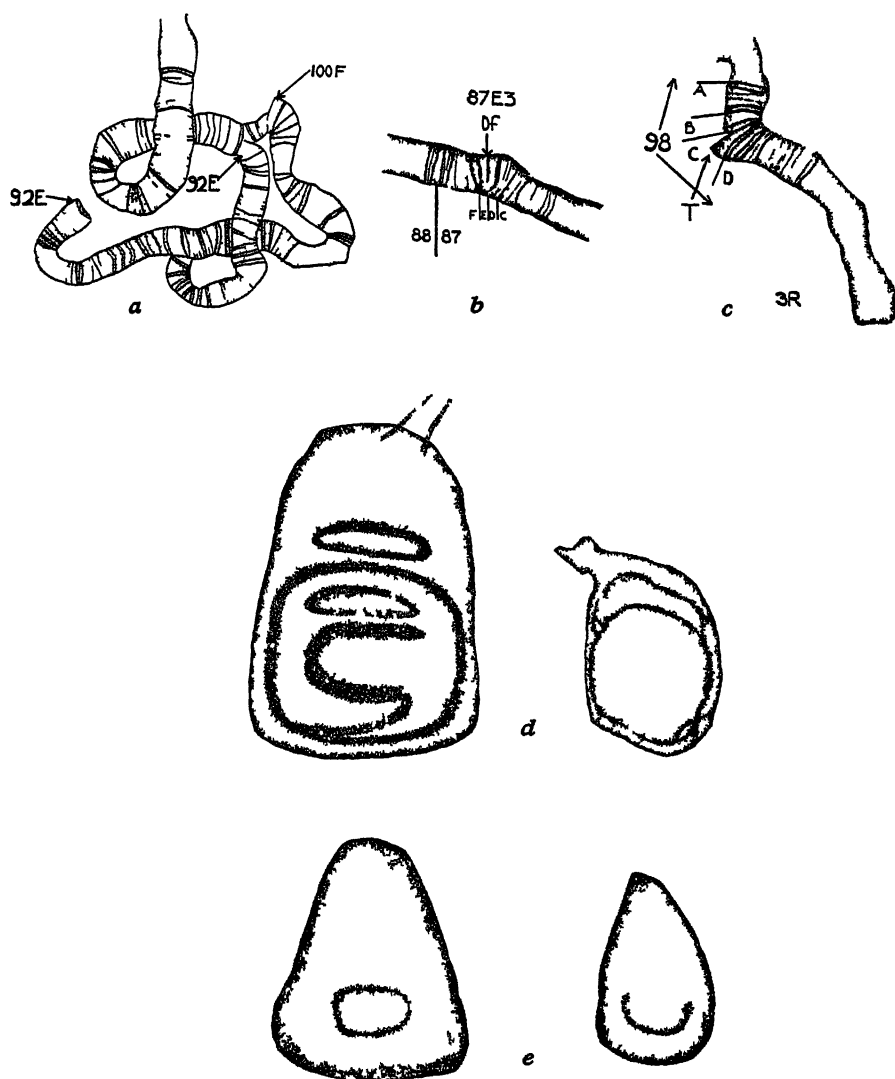


Fig 5 (a) Inversion, similar to In3RC, extending from band 92E to band 100F, present in tetraltera  $\times 1500$  (b) Heterozygous deficiency for band 87E3 from tetraltera stock  $\times 1500$  (c) Heterozygous translocation or transposition at band 98C1 from tetraltera stock  $\times 1500$  (d) Dorsal mesothoracic discs from mature tetraltera larva 1 hour before pupation left normal disc, right, smaller, less differentiated disc (e) Dorsal mesothoracic discs from 72-hour larva left more or less normal disc right, smaller, less differentiated disc



## PLATES

PLATE 10

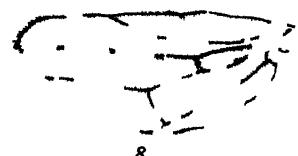
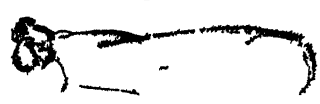
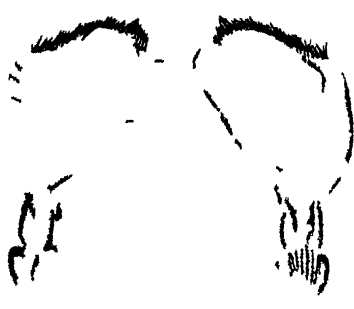
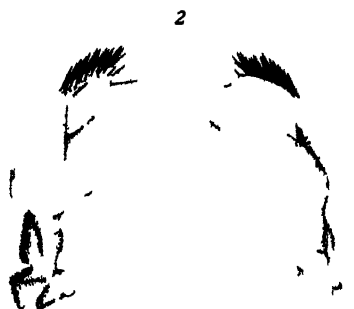
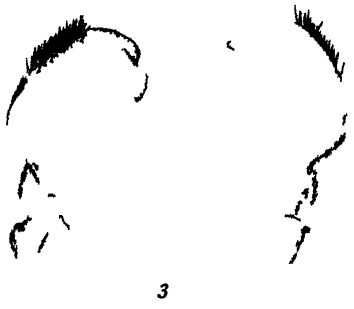
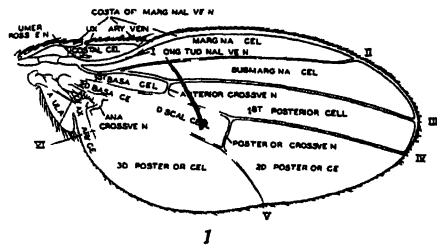
Fig 1 Diagram of dorsal view of normal wing of *Drosophila melanogaster* I, costa, II, radius, III, medius, IV, cubitus, V, analis veins (after Bridges, D I S , 9)

Fig 2 Normal haltere of *Drosophila melanogaster* left, ventral view, right, dorsal view (after Astauroff)

Figs 3-5 Consecutive grades of tetraptera halteres left, ventral view, right dorsal view (after Astauroff)

Figs 6-8 Higher grades of tetraptera halteres, dorsal views (after Astauroff)

Fig 9 Left side of thorax of tetraptera fly showing hairy outgrowth of dorsal side of metathorax (after Astauroff)



## PLATE 11

Fig. 10. Wing mount of tetraltera Ia wing; analis and cubitus vein remnants, medius vein interrupted.

Figs. 11-13. Wing mounts of tetraltera Ib wings.

Fig. 14. Tetraltera fly, left wing type Ic.

Figs. 15-17. Tetraltera flies, right wings type Ic. Note veins on the inflated sac

Figs. 18-19. Wing mounts of two types of wing aberrations grouped together as tetraltera Id.



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THE STRUCTURAL CHARACTERISTICS AND  
NUCLEAR PARASITES OF SOME SPECIES  
OF TRICHONYMPHA IN TERMITES

BY

HAROLD KIRBY

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# THE STRUCTURAL CHARACTERISTICS AND NUCLEAR PARASITES OF SOME SPECIES OF TRICHONYMPHA IN TERMITES

BY  
HAROLD KIRBY

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## INTRODUCTION

IN PROBABLE agreement with all other Zoömastigophora, flagellates of the genus *Trichonympha* are capable of only asexual reproduction. The possibility of differentiating taxonomic units in asexual organisms is a matter of special interest, because of the recent tendency of some geneticists to doubt the applicability of the species concept to such forms. Consideration of the more complex polymastigote and the hypermastigote flagellates does, I think, bring out the inexactness of the contention that the species is here no more than an arbitrarily delimited section of a continuously variable series. To be sure, here as elsewhere, species definition is not always certain or positive; the point is that recognition of what seem to be natural units presents no greater difficulties than in sexually reproducing protozoa.

Among hypermastigote flagellates, as these are known at present, there is no genus more favorable for taxonomic study than *Trichonympha*. Its advantages lie in its highly developed structural characteristics, its stability, and its relatively large number of known species. The morphological characteristics, upon differences in which distinction of taxonomic units may be based, are as complex as in any protozoa. That *Trichonympha* is an ancient and stable genus is indicated by its distribution, together with its host specificity. There are species of the genus in *Cryptocercus punctulatus* (Cleveland *et al.*, 1934) and in three of the four families of termites that contain any hypermastigotes (Kirby, 1932). (The fourth family consists of only the one species, *Mastotermes darwiniensis*.) There is reason for believing that *Trichonympha* has been distributed through these hosts not by secondary infestation but by accompanying them in phylogenetic development (Kirby, 1938).

In order to prepare the way for taxonomic analysis of the genus, information must be accumulated, in as much detail as possible, concerning every characteristic of the organisms that it is possible to study. In this paper there is described the morphology of certain species of *Trichonympha*; and in some instances my earlier accounts (1932) are corrected and supplemented. All known species of the flagellates in termites of the family Kalotermitidae have been studied again, for completion and verification; and revised diagnoses are given. To the list of thirteen species previously known in termites five new species are added.

I have studied the achromatic figure in division of several species, primarily in order to obtain a clearer understanding of the nature of certain structures in the non-dividing flagellate. Some comparisons have been made with the division process in the species of *Trichonympha* of *Cryptocercus*, described by Cleveland *et al.* (1934). No attempt has been made to give here a complete account of the division process.

In the course of the investigation my attention has been drawn to the nuclear parasites. Mention of some of these was made at the meeting of the Third International Congress for Microbiology in September, 1939. The abstract published in the Proceedings (Kirby, 1940) necessarily omits descriptive details. A more extended

account of nuclear parasitism in *Trichonympha*, with illustrations, was given in the chapter on organisms living on and in the bodies of protozoa, published in *Protozoa in Biological Research* (Kirby, 1941). In preparation of the present account a study was made of nuclear parasitism in all species of *Trichonympha* in my collection.

Some of the material used was turned over to me by Professor L. R. Cleveland; this had been collected in Java, Ceylon, and Australia. Other material was obtained by myself in Africa, Madagascar, and Java. Some termites from Florida containing *Trichonympha* were sent to me by Professor E. M. Miller, Professor A. E. Emerson, and Mr. V. H. Dropkin. I am indebted to Professor Emerson and Professor S. F. Light for information about, and determination of, the termites. Technical assistance in the study of the flagellates has been given by Mrs. Joy Barnes Cross and Mr. Jacob Frenkel, through support by the Research Board of the University of California. For financial support of the work acknowledgment is also due to the John Simon Guggenheim Memorial Foundation, the Work Projects Administration, Official Project 165-1-08-73 unit C 1, the National Youth Administration, and Sigma Xi. Acknowledgment of aid from various persons in my collecting work in Africa and Java has been made in the first paper in volume 45 of this series. In collecting certain material of *Trichonympha* in Madagascar I was aided by Mr. Gregoire Olsouffeff; and in Mauritius by Mr. Jean Vinson. Many of the drawings of the flagellates and of the nuclear parasites were made by Miss Agnes Hensley. Others, particularly those of *Trichonympha turkestanica*, were made by Mr. Carl M. Stover. Mrs. Frieda Abernathy prepared the diagrams of division of *Trichonympha chattoni*. Some diagrams of the parabasal apparatus were made by Mrs. Mabel L. Kirk.

## TRICHONYMPHA IN TERMITES OF THE GENUS ANACANTHOTERMES

### *Trichonympha turkestanica* Bernstein

(Pl. 20, fig. 103; pl. 21, figs. 104-118; pl. 22, figs. 119-137; fig. E, 5; fig. H, 1-6)

*Trichonympha turkestanica* Bernstein, 1928, Arch. Protistenk., 61:11, pl. 1, figs. 1-8, text figs. 1, 2.

*Deltotrichonympha numidica* Duboscq, Grassé, and Rose, 1937, C. r. Acad. Sci., 205:575; Duboscq and Grassé, 1941, C. r. Acad. Sci., 213:367.

?*Trichonympha fletcheri* De Mello, 1941, Arqu. Esc. Méd.-Cirúrg. Nova Goa, (A), fasc. 15:1, pls. 1, 2.

Type host.—*Anacanthotermes murgabicus* Vasiljev. Turkestan.

Additional hosts.—

*Anacanthotermes ochraceus* Burmeister. Egypt.

T-1007. Sharkiya Province. (Slides TP-1012:23, 24, 33; TP-1013:18.)

?*Anacanthotermes macrocephalus* Desneux. Sargodha, India.

?*Anacanthotermes vagans* Hagen. Muscat, Gallail, Arabian Peninsula.

?*Anacanthotermes viarum* (Koenig). Coimbatore, India.

**Diagnosis.**—(From type host, after Bernstein): length 180 (140-250) $\mu$ , width 72 (43-115) $\mu$ ; flagellated region occupies  $\frac{2}{3}$  to  $\frac{3}{4}$  of the body length, or more; flagella rather short, longer at posterior end of region; number of plates, about 95-98; ectoplasmic layer thick, with granules in the outermost layer in the anterior part, throughout the ectoplasm in the posterior part of the flagellated region; parabasal apparatus not observed; nucleus approximately in middle of body, ratio of nuclear diameter to body length 1.0:6.7 (diameter 27 $\mu$  \*); pre-nuclear endoplasmic inclusions; small, rounded, deep-staining granules abundant in region just posterior to rostrum. (From *Anacanthotermes ochraceus*): length 183 (125-270) $\mu$ ,\* width 91 (69-125) $\mu$ ; dimensions of rostrum: length in center 10-13 $\mu$ , diameter at base of cap 9-11 $\mu$ , diameter at base of collar 12-18 $\mu$ ; length of flagellated region 119 (70-170); ratio of flagellated to non-flagellated region from

\* All original measurements given in this paper were obtained from specimens fixed in Schaudinn's, Flemming's, or Hollande's fluid.

1.27:1.00 to 3.43:1.00, averaging 1.86:1.00; length of flagella approximately 10–50 $\mu$ , longer ones at posterior part of flagellated zone; ectoplasm of flagellated region about 6–8 $\mu$  thick, in three layers, peripheral granules present between plates in outermost ectoplasm, in posterior half peripheral granules, of another sort, distributed throughout ectoplasm; number of plates anterior to circular fissure 52–57, posterior to circular fissure 89–102; parabasal cords 42–68, twisted and irregularly sinuous, all entering the peripheral endoplasm at various points from about  $\frac{1}{2}$  to  $\frac{1}{2}$  the distance from the nucleus to the circular fissure to a point somewhat posterior to the nucleus, connected by filaments to the region at the base of the rostrum; many of the cords that begin more anteriorly come in contact with the nuclear membrane, then extend free posterior to it; ring present in peripheral endoplasm a short distance posterior to the mantle of basal granules; distance from anterior end of body to anterior end of nucleus 70–130 $\mu$ ; diameter of nucleus 19  $\times$  22.5 (15  $\times$  20–22  $\times$  30) $\mu$ , chromatin in a compact mass of coiled strands, nucleolus often 2–3 $\mu$ , sometimes larger, typically present in outer part of chromatin mass, sometimes also a peripheral, chromatic structure in form of a straight or twisted rod; anterior endoplasmic inclusions; small granules abundant just posterior to rostrum and in central part of endoplasm.

It has not been possible to compare the Egyptian material with specimens from *Anacanthotermes murgabicus*, and because of the incompleteness of Bernstein's description there cannot be absolute certainty that my specimens are specifically identical with hers. I have preferred to regard them as the same, however, because of the close resemblance in all features that she did describe. She stated that the "Suspensorialapparat" (parabasal apparatus) is absent, and that fact, if so, would certainly constitute a specific, if not a generic, difference. I think it highly probable, however, that a parabasal apparatus was present in her specimens, but was overlooked. It might be found that there is some difference in morphology between the parabasal apparatus of the trichonymphas from *A. murgabicus* and *A. ochraceus*; then the Egyptian form would be named *T. numidica* (Duboseq, Grassé, and Rose). Those authors (1937) stated that *T. turkestanica* probably belongs to the genus *Deltotrichonympha* Sutherland, and assigned their species to that genus; but that generic assignment is certainly incorrect. The differences are obvious when specimens are compared; and they are likewise unmistakable if Sutherland's figures (1933) are compared with Bernstein's. Duboseq, Grassé, and Rose gave no description of this flagellate of *A. ochraceus*, except for mention of the proportionately long flagellated zone, the parabasal apparatus, and the ring (ceinture lipidique) at the posterior end of the flagellar lines.

Duboseq and Grassé (1941) published a further account of the flagellate, but I have been unable to obtain the paper because of war conditions. An abstract published in Biological Abstracts (16:15581) referred to their conclusion that the flagellar apparatus of *Trichonympha* is similar to that of *Spirotrichonympha*, and to observations on the extension of the parabasal apparatus to or beyond the end of the flagellar zone.

I reported before (1932) having found *Trichonympha*, which corresponded in all observable characters to *T. turkestanica*, in alcoholic specimens of *Anacanthotermes macrocephalus* from the collection of Professor Light. Professor Emerson has recently sent me alcoholic specimens of *Anacanthotermes macrocephalus* Desneaux, including metatype material collected at Sargodha, India; *A. turkestanicus* Jacobson, cotype material from Turkestan; *A. vagans* Hagen, collected at Muscat, Gallail, on the Arabian Peninsula; and *A. viarum* from Coimbatore, S. India. The gut contents of these termites were mounted unstained in glycerine jelly. In all except *A. turkestanicus*, of which only a fragment of one specimen was available, *Trichonympha* was present and in a relatively good state of preservation, considering the nature of the material. The dimensions of the body came within the range of *Trichonympha turkestanica*. In *Anacanthotermes macrocephalus* some specimens were found which, like those observed in 1932, had a length exceeding 200 $\mu$  and a



width of only 40–50 $\mu$ ; but in other termites of the same species flagellates of more normal shape, 165–190 $\mu$  by 42–75 $\mu$ , were present. In the other termites the body sizes were similar. All of them agreed closely in the dimensions of the rostrum: length in center 11–15 $\mu$ , diameter at base of cap 10–13 $\mu$ , diameter at base of collar 19–23 $\mu$ . Only in the last-named dimension is there an appreciable difference from the Egyptian material; and that is not significant in view of the great difference in the mode of preparation. The rostrum corresponded in all details with that in the Egyptian specimens, including the central rod within the anterior part of the rostral tube. The plates, layers of ectoplasm, and endoplasmic granules were very distinct in the alcoholic specimens, and all agreed in every observable detail with the corresponding structures in *T. turkestanica*. Peripheral granules were also present in the posterior part of the collar region and between the plates. The nucleus had the same position, structure, and size range in all the trichonymphas from *Anacanthotermes*. In the alcoholic material it was not possible to see anything of the parabasal apparatus. In view of this, and of the type of material to which my studies have been restricted, the termites from India and Arabia have been listed above as doubtful hosts of *Trichonympha turkestanica*. To me it seems probable, however, that the species is *T. turkestanica* in all these species of *Anacanthotermes*. Thus the situation is like that in *Zoötermopsis*, *Glyptotermes*, and *Reticulitermes*. Trichonymphas that, in so far as structural characteristics go, seem to belong to the same species occur in numbers of related hosts.

The rostral tube (pl. 21, fig. 104) is a cylindrical structure that maintains for most of its length its posterior diameter of about 3 $\mu$ , then decreases rapidly in diameter in its most anterior part, before it meets the crescentic bodies (pl. 21, figs. 104–108). These bodies are two in number, well defined and exactly symmetrical, appearing from one aspect (pl. 21, fig. 104) as a rod across the anterior end of the rostral tube, and from the other (pl. 21, fig. 105) in optical section, as two granules. Anterior to these crescentic bodies is a hemispherical mass of dense cytoplasm, and over that is a well-defined, membranous inner cap (pl. 21, fig. 104). This inner cap has a diameter at its base of 4–5 $\mu$ . Its margin coincides with the outer edge of the innermost zone of ectoplasm; but in heavily stained material it may seem to be more extensive.

In the center of the rostral tube, beginning between and just posterior to the crescentic bodies, and extending for a length of 4–5 $\mu$ , is a rod (pl. 21, figs. 104–108). This central rod stains very deeply with iron-haematoxylin after Schaudinn's fluid. Much attention has been devoted to sections through the rostrum giving both side and end views of the crescentic bodies and the central rod. It was necessary to ascertain whether in this species the rod turns anteriorly and joins one of the crescentic bodies, as described by Cleveland *et al.* (1934) in *Trichonympha* of *Cryptocercus punctulatus*.

According to Cleveland's account, the crescentic bodies represent the two centrioles. One of these is extended posteriorly in the rod in the anterior part of the rostral tube; this rod, with the crescentic body to which it is attached, is the old, elongate centriole whose distal end has functioned in the production of the achromatic figure of the preceding mitosis. The other crescentic body, which is not attached to the rod, is the proximal end of what will be the new elongate centriole; it does not develop a rod until the onset of cell division.

According to my observations, in *T. turkestanica* the rod at its anterior end does not show a direct connection, at least in its heavily stainable substance, with either one of the crescentic bodies; its anterior end is equidistant between the two, and the fine membranous or fibrous extensions that may appear between this end and the crescentic bodies are symmetrically arranged, as concerns both of them (pl. 21, figs.

105-108). A strict symmetry is thus present in the structure of the rostrum, the rod being in the center of the figure. Nevertheless, in separation of the rostral tube into two halves the rod goes with one half, and a new one develops in relation to the other half, as is described below (p. 222).

As Cleveland *et al.* (1934) pointed out, I was in error in the description (1932) of a single hemispherical granule, called the blepharoplast, at the anterior end of the rostral tube of species of *Trichonympha*. The description then given was a consequence of observing overstained material, in which the inner cap and its contents give that appearance; but it is more difficult to observe the crescentic bodies in *T. collaris*, *T. campanula*, and *T. sphaerica*, the species to which I then devoted most study, than in some other species. The significance of the structures at the anterior end of the rostral tube of *T. chattoni* seen in optical section as a pair of granules, which Duboscq and Grassé (1927b) noted and regarded as possibly an optical section of a ring or disc, was not then understood. I have seen paired structures of this type in all species of *Trichonympha* studied in preparation of this paper.

The rostral tube consists of two halves which are not separable optically until the onset of division. When heavily stained with iron-haematoxylin the rostral tube appears as a black, homogeneous cylinder; with better differentiation it shows a granular structure (pl. 21, fig. 104). The granules are arranged in transverse circles. It thus appears to consist of basal granules, which are not "fused" as I formerly supposed, but are separately imbedded in a matrix substance. Within the rostral tube is endoplasm largely free of granules.

According to Bernstein's account of the rostrum, the "rostral tube" consists not only of the rostral tube as recognized by Kirby (1932) and Cleveland (1934), but also of the innermost zone of ectoplasm. Bernstein described a ring-shaped thickening at the base of the rostral tube anterior to the circular fissure, and considered this to represent a sphincter which closed the opening and kept the granules of the endoplasm out of the tube. In some specimens I have seen in this position a ring much more slender than the one she represented; but there is no such constriction of the rostral tube.

Bernstein stated that posterior to the circular fissure there is a larger ring, and she wrongly considered it to correspond to the centrobalepharoplast of Kofoid and Swezy. As shown in her figures, this ring occupies a large part or all of the middle (her inner) zone of ectoplasm posterior to the circular fissure. In my preparations this area, as the parts posterior to it, is occupied by the roots of flagella. It is probable that the ring, named and figured as the centrobalepharoplast by Bernstein, does not exist; its description may be based on a deposit of haematoxylin in that form, as may happen in preparations.

The zone of thick ectoplasm extends posteriorly more than half the length of the body (pl. 20, fig. 103; pl. 21, fig. 112). It usually maintains a thickness of 6-8 $\mu$  from the circular fissure to near its posterior limit; then it tapers rather abruptly. Of this zone of thick ectoplasm in the flagellated region, the anterior half differs in appearance from the posterior half (pl. 20, fig. 103). In an optical section of a whole mount (pl. 22, fig. 119), four ectoplasmic layers can be distinguished in this anterior portion. First is the thin, dense-appearing innermost layer in which the basal granules are situated. Then there is a clear layer, which has a width of about a third of the ectoplasm, and is crossed by very distinct flagellar roots. Outside this is a layer which, in certain preparations, appears very dense; it reaches to within a micron of the periphery. The outermost layer is narrow and contains numerous granules. The roots of the flagella pass through clear layer 2 in a direction more or

less vertical to the longitudinal axis of the body. At the outer limit of layer 2 they bend sharply, and pass through layers 3 and 4 obliquely posteriorly. The dense-appearing material of layer 3 becomes somewhat narrower as it passes posteriorly, leaving more space for the peripheral granules, and at the posterior limit of the anterior half of the thick ectoplasm it ends rather abruptly in a truncate or rounded form.

In the posterior half of the thick ectoplasm the division into distinct layers is not so evident. Layer 3 is not so clearly differentiated from layer 2, although the flagellar roots change their direction at the limit between them; and there is no distinction of a thin outermost layer.

There are two types of granule in the ectoplasm of the flagella-bearing region (pl. 22, fig. 122). One type is small, more or less spheroidal in form, and is numerous in the outermost part of the ectoplasm (pl. 22, fig. 127). In the ectoplasm of the anterior half of the flagellated region, and in the rostrum (pl. 21, fig. 104) it is the only type. In the rostrum, where these granules are limited to the posterior part, their distribution is much the same as that of the peripheral granules of *Trichonympha collaris*. They are situated between the plates, usually lying closer to the left side, that is, in the right side of each space between the plates (pl. 22, figs. 124, 126). This arrangement is as described by Bernstein, but her plate 1, figure 1, shows the opposite arrangement. The granules are occasionally in the middle of the space between the plates, or near the right side of the plates; and there is no such regularity in the series of granules as there would be in basal granules of flagella, for which Bernstein mistook them.

The granules of the second type (pl. 22, figs. 122, 129) occur mostly in the posterior half of the flagella-bearing ectoplasm. They are considerably larger in size than the other granules and have for the most part an elongate form. Some show binary fission. They are often particularly abundant in the clear layer of ectoplasm adjacent to the innermost layer, and they sometimes extend forward for a short distance beneath dense-appearing layer 3 of the anterior half. They occur also in the outer ectoplasm in the posterior half, but never in layer 3 of the anterior half. In Schaudinn-protargol preparations the granules of the first type are often completely unstained, or are faint, whereas those of the second type are deeply impregnated. In such preparations the distinction of the two types is clearly evident. Both types are present in the ectoplasm of all specimens.

In size and distribution the peripheral granules of *Trichonympha collaris* resemble the smaller granules; but they are somewhat larger and many have a rod form. There are no granules in *T. collaris* like the second type granules of *T. turkestanica*. In the ectoplasm of a few specimens I have seen an elongated symbiont, pointed at the ends, which stains deeply with iron-haematoxylin (pl. 22, figs. 125, 126). The organisms range in length from 3.5 to 5.5 $\mu$ , and those of the same length vary in thickness. Some are stout spindles, some swollen rods. Often there are no more than 1 or 2 spindles in a specimen; the maximum number seen was 12. They are present only in the ectoplasm of the flagella-bearing region, between the plates.

Cross sections through the rostrum (pl. 21, fig. 107) and through the middle of the body (pl. 21, fig. 115) show the radial arrangement of the flagellar plates. They do not extend in a straight radius, but are somewhat curved. The plates are located in the thick outermost layer of ectoplasm (layer 3, and 4 where that is differentiated). In the rostrum the number varies from 52 to 57; posterior to the circular fissure there are 89-102, the mean lying between 94 and 95. In the region posterior to the circular fissure at the inner edge of each plate there is an enlarged or deeper-

stained riblike structure (pl. 22, figs. 127-129; pl. 21, fig. 114). Inward from this extend the flagellar roots, lying directly in the continuous clear layer of ectoplasm, with no stained enclosing matrix. In the innermost layer of ectoplasm, as seen in transverse section, each root passes through a rodlike enlargement. Across the inner parts of these enlargements is a well-defined line, which seems to represent an optical section of a thin membrane at the inner boundary of the dense inner layer of ectoplasm, in which the enlargements lie. The enlargements seem to pass through this membrane for a very short distance. At their innermost ends are the basal bodies. A membranelike structure separating the layer of basal bodies from the thick zone of ectoplasm is present in other species of *Trichonympha*; it was shown in *T. campanula* by me (Kirby, 1932, pl. 21, fig. 4), although not discussed in the text.

The basal bodies, except in the rostrum and the most anterior part of the flagellated region, appear as rods in many preparations (pl. 21, fig. 109). The rods lie obliquely or perpendicularly to the ends of the inner enlargements of the roots. Some rods appear solid, about  $\frac{1}{2}\mu$  long; others show two deep-stained granules, constituting the ends of the rods, between which a matrix substance extends (pl. 21, fig. 114). In some preparations the granules appear entirely separate, the interconnecting substance not being stained (pl. 21, figs. 110, 111). The basal bodies have been revealed particularly well in certain protein silver (protargol) preparations (Bodian, 1937), restained after original staining in iron-haematoxylin following Schaudinn's fixation.

In the anterior part of the flagellated region of the body, the basal bodies are incorporated into a compact, deep-staining substance, as in the rostral tube, with which this substance is continuous (pl. 21, fig. 104). Posteriorly the rods or granules appear separately. The basal bodies appear to be included in an actual membranous mantle (pl. 21, fig. 111). At its posterior limit there is a well-defined line, coinciding in position with the last row of basal bodies. The substance of the mantle is distinctly darker than that of the cytoplasm posterior to it, or that external or internal to it.

Fibrils interconnect the basal bodies both longitudinally and transversely (pl. 21, fig. 114). A rather indistinct longitudinal fibril appears in some protein silver preparations, running parallel to each plate and the corresponding basal bodies (pl. 21, figs. 113, 114). It is situated slightly inward from these bodies in the outer ectoplasm, and there seems to be a short transverse fibril connecting one granule of each basal rod to the longitudinal fibril.

At the posterior end of the flagellated region the diminution in thickness of the endoplasm which takes place in the region between the end of the mantle of basal granules and the outer ends of the plates is rather rapid (pl. 20, fig. 103; pl. 22, figs. 135-137). The layer of basal bodies terminates at the posterior limit of the thick ectoplasm. The roots of the flagella extend obliquely posteriorly; so that the outermost part of the flagella-bearing plates are farther posterior. Posterior to the flagellated region the ectoplasm has a varying thickness, often about  $1.5\mu$ , and is delimited from the endoplasm by a sharp boundary, which sometimes resembles a membrane. The posterior ectoplasm has a finely granular texture in fixed material, and is free of larger granules and wood particles like those that occur in the endoplasm.

The parabasal apparatus (pl. 20, fig. 103; pl. 21, fig. 112) consists of numerous parabasal cords, each continued anteriorly by a filament to the region of the base of the rostral tube. The anterior ends of the parabasal cords (the stouter parabasal structure, as opposed to the free filament) all lie close to the boundary between the

ectoplasm and endoplasm at various points in the flagellated region (pl. 21, figs. 113, 114; pl. 22, fig. 122). None begin in the anterior part of the body; the most anterior point of origin is usually about  $\frac{1}{3}$  to  $\frac{1}{2}$  the distance from the nucleus to the circular fissure (pl. 21, fig. 112). Most but not all of the cords commencing in this anterior position come in contact with the nuclear membrane before passing on posteriorly. Other cords, beginning more posteriorly, some even posterior to the nucleus, generally do not come in contact with the nuclear membrane. Whatever the point of origin, the cords tend to end at the same level posteriorly; thus their length varies considerably. As noted by Duboseq, Grassé, and Rose, the cords terminate posterior to the nucleus, but anterior to the posterior extremity of the flagellar lines.

The approximate number of parabasal cords is 42 to 68, with the mean number 50. They are twisted and sinuous in form, but are not turned in regular spirals. Along one edge of each cord is a deep-staining filament, like that in the parabasals of *T. chattoni* (Duboseq and Grassé, 1927b) and *T. collaris* (Kirby, 1932). This filament can be seen clearly in Schaudinn-iron-haematoxylin preparations. In cross section it appears as a granule within the periphery of the circular cord (pl. 22, fig. 129). In protein silver material two edges of the parabasal cord are blackened, and the filament is not distinguishable. At the anterior end of the parabasal cord is abruptly narrowed or simply rounded. There is no gradual taper of great length, but individual cords vary in this respect.

From the anterior end of each cord the parabasal filament alone continues to the base of the rostral tube. The free parabasal filaments are located in the peripheral endoplasm very close to the layer of basal bodies. The course of the parabasal filaments is roughly but not altogether parallel to the plates; and is more or less unevenly sinuous. The free filament was unstained in the protein silver preparations; for that reason, only the cordlike part of the parabasal is shown in plates 21 and 22, figures 112 and 122. The latter substance stained intensely in these preparations.

The fact that the cordlike part of the parabasal bodies always begins close to the layer of basal granules, and that the filaments are always peripheral in the endoplasm, is a notable characteristic of the flagellate. Some of the parabasal cords extend for a short distance vertical to the layer of endoplasm, then bend sharply and pass posteriorly. Thus the parabasals appear not to be loosely suspended from the rostrum, but the filaments are somehow maintained for their full length in a peripheral position.

I have not observed within the rostral tube a parabasal lamella such as Cleveland *et al.* (1934) found in *Trichonympha grandis*.

Characteristic of this species of *Trichonympha* alone among those I have studied is the presence of a ring in the endoplasm a short distance posterior to the mantle of basal granules, between this and the ends of the plates (pl. 20, fig. 103; pl. 21, fig. 112; pl. 22, figs. 134-137). The ring is a cordlike homogeneous structure with a thickness often of about  $1\mu$ . It is often irregularly bent in its course; sometimes it is discontinuous. It is stained, although not very deeply, in iron-haematoxylin after fixation in Schaudinn's and Flemming's fluids. It is unstained by Delafield's haematoxylin or protein silver after these fixatives.

Bernstein did not describe this ring in *T. turkestanica*, but it is not unlikely that she overlooked it, along with the parabasal bodies. Duboseq, Grassé, and Rose (1937) noted its presence immediately beneath the ectoplasm, and termed it "ceinture lipidique."

Between the flagellar plates, and extending posteriorly beyond them, are certain

membranes and fibrils. I have been unable to determine the connections and interrelations of all these structures, but they seem to belong in three or four categories. Between each two plates and parallel to them is a membrane, delicate and often not visible at all, extending from the outermost ectoplasmic boundary to the boundary of the endoplasm (pl. 21, fig. 114; pl. 22, fig. 130). The membrane is sinuous in its course, sometimes closer to one plate, sometimes to another. The cytoplasm on one side of it may appear denser than that on the other side (pl. 22, fig. 131), and the peripheral granules of the smaller size are confined to one side, between the membrane and the plate to the right of it. A fibril (pl. 22, fig. 121) is visible between each two plates, beginning where the denser anterior part differentiates from the posterior part of the flagella-bearing ectoplasm. At its origin anteriorly (pl. 22, fig. 120) this fibril lies at the innermost part of the ectoplasm, then curves obliquely outward to the outermost ectoplasm, and becomes applied to the plate on the left side. It cannot be traced posteriorly beyond this point of contact. Between each two plates, at a level near their posterior ends, a fibril arises in a deeper layer of ectoplasm, extends peripherally some distance, then bends inward again to come in contact, or close to contact, with the ring (pl. 22, figs. 134-137). There is also a fibril passing posteriorly over the ring without the inward bend; it appears to join the other fibril anteriorly and posteriorly. Those fibrils which establish contact with the ring continue in the deeper ectoplasm to the posterior end of the body, sometimes uniting with one another in pairs or groups (pl. 22, fig. 137).

The chromatin of the interphase nucleus (pl. 21, fig. 116) is in the form of a rather compact mass of coiled strands, equally dense in all parts; and in fixed material, at least, there is a clear zone containing small granules between the chromatin mass and the nuclear membrane. In some nuclei the strands are more or less broken up into interconnected fragments and granules.

Many of the nuclei contain two bodies, of differing kind, in addition to the chromatin strands. One is a spheroidal nucleoluslike body, located usually in the outer part of the mass of chromatin strands, but not peripheral to them; as a rule there are some strands between it and the nuclear membrane. This body varies much in size, but frequently measures 2-3 $\mu$ . However, it may reach a diameter of 8 $\mu$ , or even 9  $\times$  12 $\mu$ , in nuclei that show no prophase changes in the distribution of chromatin. Occasionally it is more or less heterogeneous, a deep-staining granule or structure of varied form being differentiated within it. In some nuclei no nucleoluslike body can be detected, but most have one and some have two or three such bodies. The other body, which cannot always be detected, is peripheral to the chromatin mass and is set off from it by a clear space. It corresponds to the "heterochromosome" of *Trichonympha campanula*, and probably to the chromatin nucleolus which is present in *Barbulanympha* species and *Trichonympha grandis* (Cleveland *et al.*, 1934). In *Barbulanympha* this body plays no role in mitosis. In *T. turkestanica* it has the form of a short rod, often appearing more or less moniliform or granular. Sometimes it is straight (pl. 21, fig. 117), more often it is curved and twisted (pl. 21, fig. 116). In protein silver preparations it is often impregnated much more deeply than the chromatin.

A few binucleate specimens of *Trichonympha turkestanica* have been found. These gave no indication of being in a division stage; except for the two nuclei, they correspond in every way to ordinary interphase flagellates. The nuclei were each in the interphase condition, placed side by side, and sometimes closely applied to one another (pl. 21, fig. 118).

The prenuclear endoplasmic inclusions are granules, many with a diameter of about 1/2 $\mu$ . In the region near the nucleus they are few and scattered; in the narrow

region just posterior to the circular fissure they are densely packed (pl. 21, fig. 104). They do not extend into the endoplasm within the rostral tube.

No spherules, like those in *T. sphaerica* and *T. chattoni*, have been seen in the posterior endoplasm.

### *Trichonympha fletcheri* De Mello

*Trichonympha fletcheri* De Mello, 1941, Arq. Esc. Méd.-Cirúrg. Nova Goa, (A) fasc. 15:1, pls. 1, 2.

Type host.—*Anacanthotermes viarum* (Koenig). Coimbatore, India.

*Diagnosis*.—(After De Mello): length 100–183 $\mu$ ; width 50–116 $\mu$ ; dimensions of rostrum: length in center 8–14 $\mu$ , diameter at base of cap 8 $\mu$ ; diameter at base of collar 19 $\mu$ ; flagellated region occupying about three-fourths of the length of the body; length of the flagella at the anterior part of the rostrum 13–15 $\mu$ , on most of the body 6–7 $\mu$ , at the posterior part of the flagellated zone 25–27 $\mu$ ; peripheral granules present between the plates; parabasal cords present (but described only in part); distance from anterior end of body to anterior end of nucleus 25–83.5 $\mu$ , majority 50–60 $\mu$ ; dimensions of nucleus 22  $\times$  28 to 27  $\times$  33 $\mu$ ; small granules abundant in a "sensory sac" occupying the prenuclear endoplasm.

In the foregoing account of *Trichonympha turkestanica* I mentioned the fact that I had seen *Trichonympha* in alcoholic specimens of *Anacanthotermes viarum* from the collection of Dr. A. E. Emerson. After the manuscript of this paper had been in press for almost a year, I received from Colonel I. Froilano de Mello a reprint of his paper in which he described *Trichonympha fletcheri* from the same termite, collected in the locality from which Emerson's specimens had been obtained.

The differences in body size of *T. turkestanica* of Bernstein, the Egyptian *T. turkestanica* of Kirby, and *T. fletcheri* are within the possible limits of variation in a species of *Trichonympha*. The lengths of the flagella should be compared in specimens examined under comparable circumstances, before the differences that seem to exist in them are considered to be significant.

As it is shown by De Mello, the rostral tube in *T. fletcheri* is shaped like a flask with its posterior section rounded out; whereas in *T. turkestanica* the tube is cylindrical and of even diameter in all except its most anterior part. At the posterior end the dimensions of the rostral tube in the two hypermastigotes are the same. In the unstained specimens from *Anacanthotermes viarum* mounted in glycerine jelly, I have been able to see the rostral tube, and other rostral structures, very clearly. The shape of the tube is the same as in *T. turkestanica*. It is probable that De Mello is incorrect in this matter, since I have not seen a tube shaped like that which he shows in any species of *Trichonympha*, and the existence in *Anacanthotermes viarum* of a second species similar to *T. fletcheri* in other respects is very unlikely.

De Mello observed the central rod in the rostral tube of *T. fletcheri*, but it is shown as more slender and as extending farther posteriorly than in *T. turkestanica*. Here again in my specimens in glycerine jelly the central rod in the rostral tube corresponds in thickness and length to that in *T. turkestanica*.

The structures at the anterior end of the rostral tube of *T. fletcheri* are evidently similar to those in *T. turkestanica*, although they are inaccurately described by De Mello. He regarded the inner cap and the mass of cytoplasm which it encloses as the blepharoplast, repeating the error which I made in 1932. He evidently observed at the anterior end of the tube some indication of the two crescentic bodies; but he had no clear concept of the shape and position of the structures, and wrote of them as "mamillary protuberances."

I have seen nothing in *T. turkestanica*, or in the glycerine-jelly specimens from *A. viarum*, that corresponds to the collar just posterior to the circular fissure described by De Mello as a new structure in *Trichonympha*. It is probable that this, like Bernstein's ring-shaped blepharoplast in the same position, is merely a deposit

of iron-haematoxylin representing no special structure. The so-called collar in *T. fletcheri* occupies a region where it would interfere with the normal arrangement of anatomical features of the flagellate, and in the glycerine-jelly specimens there is no irregularity in arrangement.

De Mello described two sort of myonemes, but his interpretation is obviously wrong. His "transverse myonemes" are the roots of the flagella crossing the clear zone of ectoplasm. I have seen no normal structure in *Trichonympha* that corresponds to the "ramified myonemes" which are said to cover the prenuclear endoplasmic sac in *T. fletcheri*, but I think I know how De Mello made the interpretation. Where the ectoplasm meets the endoplasm there are rows of basal bodies, and when these are focused on, one sees in the glycerine-jelly specimens lines of refractile structure running in oblique and crossing directions. Actually there is no meeting of the lines; an impression of ramification might be gained, but this error is readily avoided by care in focusing.

In considering the peripheral granules as basal granules of the flagella De Mello made the same mistake as Bernstein. In *T. fletcheri* the peripheral granules of the second type (p. 190) are present as in *T. turkestanica*; these are the "rough and coarse siderophil granules" which obscure the "transverse myonemes" in the posterior part of the flagellated zone.

De Mello failed to understand the nature of the plates; he observed them, but described them as "longitudinal surface ridges," which appear as flattened ribbons. The actual surface ridges are, of course, at the surface (they do not "belong to the inner layer of ectoplasm") and are between the plates. He made very incomplete observations on the parabasal cords, which are undoubtedly more numerous and extensive than he supposed. In common with Bernstein, he did not describe the ring; the failure of these authors to describe that structure, which is present in the Egyptian specimens, does not mean that it was absent in their material. It is not always easy to observe.

De Mello stated that there is no "heterochromosome" in the nucleus; but a rounded nucleolus, which he did not mention, is present in the glycerine-jelly specimens from *A. viarum*.

The resemblance between *T. fletcheri* and *T. turkestanica* was commented on by De Mello. Unfortunately, the description of *T. fletcheri* is not given in accurate detail, and the interpretation of what observations could be made is deficient in exactness. Consequently, I cannot give a decision on the relationship between that species and the flagellate of *Anacanthotermes ochraceus* which I have considered to be *T. turkestanica*. The resemblance in morphology is probably closer than De Mello supposed; he was hampered, of course, by having available only the incomplete account of *T. turkestanica* by Bernstein. A point in favor of differentiation is the geographical separation of the hosts, but other flagellates that are indistinguishable one from another in structure have been found in termites that are even more distantly separated.

#### TRICHONYMPHA IN TERMITES OF THE FAMILY KALOTERMITIDAE

The family Kalotermitidae as here referred to is restricted according to the most recent arrangement by Emerson. It corresponds only to part of the family Kalotermitidae Banks as I understood it in my previous paper on *Trichonympha*. The genera of living termites in the restricted family are *Kalotermites*, *Neotermites*, *Paraneotermites*, *Rugitermites*, *Procryptotermes*, *Cryptotermes*, *Eucryptotermes*, *Glyptotermes*, and *Calcaritermites*.



**Trichonympha chattoni** Duboseq and Grassé

(Fig. A, 1-6; J, 1-14; pl. 12, figs. 6-16)

*Trichonympha chattoni* Duboseq and Grassé, 1925, C. r. Acad. Sci., 180:478; 1927a, O. r. Soc. Biol., 96:92, fig.; 1927b, Arch. Zool. exp. gén., 66:465, figs. 6-9, pls. 17-19, figs. 22-32. Kirby, 1932, Univ. Calif. Publ. Zool., 37:396, 445, pl. 22, fig. 10, pl. 30, figs. 59-60.

*Type host*.—*Glyptotermes indipennis* Froggatt. Australia.

T-510. (Cleveland-Hill.) New South Wales, about 50 miles north of Sydney. (Xenosyntype slides TP-510:48, 53, 89, 11.)

*Additional hosts*.—

*Glyptotermes brevicaudatus* Haviland. Java.

T-4533. Bandjar. (Homosyntype slides TP-3217:15, 5.)

*Glyptotermes brevicornis* Froggatt. Australia.

(This species includes the termites listed by Kirby, 1942, under *Foaina delicata* and *F. hilli*, as *Glyptotermes dubius* and *G. perangustus*. See Hill, 1942.)

T-511. (Hill 3232. Cleveland.) Ravenhoe, Queensland (Homosyntype slides TP-511:12, 57.)

T-512. (Cleveland-Hill.) Sydney. (Homosyntype slide TP-512:3.)

*Glyptotermes ceylonicus* Holmgren. Ceylon.

T-314. (Cleveland-Collier.) (Homosyntype slide TP-265:1.)

*Glyptotermes contracticornis* (Snyder). Costa Rica.

(Referred to by me, 1932, as *Kalotermes*. Assignment to *Glyptotermes* by Emerson *in litt.*)

T-132. Cartago. (Homosyntype slides TP-72:3, 17.)

*Glyptotermes montanus* Kemner. Java.

T-324. (Cleveland-Collier.) (Homosyntype slides TP-262:13, 48, 78.)

*Glyptotermes neotuberulatus* Hill. Australia.

T-513. (Hill 3194.) Australian Capital Territory. (Homosyntype slide TP-513:2.)

*Glyptotermes parvulus*. Sjöstedt. Gold Coast.

T-301. (Silvestri.) (Homosyntype slide TP-318:1.)

*Glyptotermes taveuniensis* Hill. Fiji Islands.

T-515. (Hill 4065. Cleveland.) (Homosyntype slide TP-515:27.)

*Glyptotermes* sp. nov. Uganda.

T-2072. Near Kampala. (Homosyntype slide TP-2023:28.)

T-2090. Budongo Forest. (Homosyntype slide TP-2028:4.)

*Glyptotermes* sp. nov. Belgian Congo.

T-3018. Near Rutchuru. (Homosyntype slides TP-2034:1; 2035:10.)

*Glyptotermes* sp. nov. Ruanda-Urundi.

T-3022. Kakitumba. (Homosyntype slide TP-2037:14.)

*Glyptotermes* sp. nov. Philippine Islands.

T-4601. (Light 1744.) Mt. Maquiling, Luzon. (Homosyntype slide TP-3285:7.)

*Kalotermes milleri* Emerson. Florida. (Description of termite by Emerson in Psyche.)

T-532. (Emerson.) (Homosyntype slides TP-535:7, 19.)

*Kalotermes schwartzi* Banks. Florida.

T-539. (E. M. Miller.) Coral Gables. (Homosyntype slides TP-546:16, 20.)

*Diagnosis*.—(From type host, after Duboseq and Grassé): length 60-125 (180) $\mu$ ; width at maximum 45-50 $\mu$ ; length of flagellated region 11-30 $\mu$ , amounting to about a sixth of the length of the body; number of body plates about 46; parabasal cords 36 (32-44), each attached to the rostrum by the chromatic filament, sinuous, extended through the endoplasm free of contact with nucleus; nucleus nearly spherical, chromatin in short curved rods or granules, excentric, spherical nucleolus of moderate size; lipid granules abundant in anterior endoplasm, spherules, many 2-3 $\mu$ , and filamentous chondriosomes in remaining endoplasm. (From type host, original): length 88 (60-120) $\mu$ ; width 56 (45-70) $\mu$ ; dimensions of rostrum: length in center 8-10 $\mu$ , diameter at base of cap 5-6 $\mu$ , diameter at base of collar 10-14 $\mu$ ; rostral tube more or less parallel-sided; length of flagellated region 21 (15-25) $\mu$ ; ratio of flagellated to non-flagellated region 0.25:1.00 to 0.39:1.00, averaging 0.31:1.00; number of plates anterior to circular fissure 24-26, posterior to circular fissure 44-51; parabasal cords varying from sinuous to closely spiraled, some or none coming in contact with nuclear membrane, most or all free and peripheral in endoplasm; chromatin in coiled strands or reticulum, spherical moderate-sized nucleolus present; distance from anterior end of body to anterior end of nucleus 15-40 $\mu$ ; nucleus 9.2 $\times$ 10.1 (7 $\times$ 9-11 $\times$ 13) $\mu$ ; abundant deep-staining granules in anterior endoplasm, zone sharply delimited anterior to nucleus; spherules numerous in postnuclear endoplasm. (From *Glyptotermes montanus*): length 102 (90-110) $\mu$ , width

50 (40–65)  $\mu$ ; dimensions of rostrum: length in center 8–10  $\mu$ , diameter at base of cap 5–6  $\mu$ , diameter at base of collar 9–15  $\mu$ ; length of flagellated region 32 (20–40)  $\mu$ ; ratio of flagellated to non-flagellated region averaging 0.43:1.00; nucleus 9  $\times$  9  $\mu$  to 10  $\times$  10  $\mu$ . (From *Glyptotermes brevicaudatus*): length 84 (65–116)  $\mu$ ; width 56 (45–70)  $\mu$ ; dimensions of rostrum: length in center 7–9  $\mu$ , diameter at base of cap 4–5  $\mu$ ; diameter at base of collar 7–10  $\mu$ ; length of flagellated region 20–25  $\mu$ , ratio of flagellated to non-flagellated region averaging 0.35:1.00; nucleus 6  $\times$  8  $\mu$  to 10  $\times$  10  $\mu$ .

*Trichonympha chattoni* has a wide distribution among termites of the genus *Glyptotermes* in various parts of the world. In all its known hosts of that genus except *G. brevicaudatus* it is associated with *Macrotrichomonas pulchra*; in that host there is a species of *Macrotrichomonas* (*M. ramosa*) that differs from *pulchra* only in the branched character of the parabasal body. *M. pulchra* has been found to occur in no termites in which it is not associated with *T. chattoni*. There are often, however, considerable differences in other respects in the faunules of termites that have these two flagellates in common.

The species is characterized by a short flagellated zone. The usual position of the nucleus is near the posterior limit of this zone (fig. A, 1), but in some specimens it has a more posterior location. In many preparations from various hosts, only specimens with the anterior nucleus were seen. In some a considerable number of *Trichonympha* with more posterior nuclei were encountered; these were found, for instance, in certain preparations from *Glyptotermes iridipennis* but not in others, in a slide from *G. neotuberculatus*, and in *Glyptotermes* spp. from Uganda and the Belgian Congo. In some of these, furthermore, the flagellated area was longer than usual.

The thick zone of ectoplasm through which the flagella extend does not taper gradually posteriorly, as is indicated in the figures by Duboscq and Grassé (1927b), but is of even width for most of its length, then narrows abruptly.

Anterior to the circular fissure the number of plates counted in specimens from *Glyptotermes iridipennis* was 24, 25, and 26, most frequently 26; posterior to the circular fissure 44 to 51 were counted. This is in agreement with Duboscq and Grassé's statement (1927b) that the number is about 46.

The parabasal cords appear first in the peripheral endoplasm at about the posterior end of the thick layer of ectoplasm. Generally, the cords extend through the endoplasm free from the nucleus, to a level varying from approximately that of the posterior end of the nucleus to a distance beyond it by about the nuclear diameter. In many specimens, but not in all, some of the cords come in contact with the nuclear membrane, usually extending along the sides of the nucleus. A few specimens have been found, in *Glyptotermes* sp. from Uganda, in which all the cords come in contact with the nucleus, but that is exceptional in the species.

The sinuosity of the cords, which appears in the figures by Duboscq and Grassé, is a feature of the species. Rarely are the cords straight or smoothly curved; usually they are turned in a spiral. The spiral may have only a few gyres, so that there appears only a loose sinuosity (fig. A, 3); but, on the other hand, there are parabasals in very close-wound spirals. The maximum closeness of spiraling was noted particularly in *Glyptotermes neotuberculatus* (fig. A, 6) and *G. montanus* (fig. A, 1). In one preparation from the former termite almost all the parabasals of *T. chattoni* were closely wound; in another, made from the same colony, many were close spirals, but others were more open, and some evenly sinuous. In single faunules from *G. montanus*, the whole range in spiraling was represented. Specimens from the type host showed similarly a variety of forms on single slides. The majority were more closely spiraled than in the specimens represented by Duboscq and Grassé; but on the same smears, and sometimes in the same flagellate, there were openly

sinuous and even almost straight parabasals. The fact that there is usually some degree of spiraling is significant taxonomically, but the degree of spiraling cannot in this instance be used for systematic purposes.

The parabasal cords may be situated rather deep in the endoplasm in their distal portion, but their anterior ends are typically peripheral. As was noted by Dubosq and Grassé, a filament continuous with that along one side of the cord (fig. A, 2) extends from the anterior end of each cord to the base of the rostrum. I have occasionally been able to see the filaments in interphase specimens, but they can be observed more readily in dividing specimens. In the interphase they lie close along the region of the flagellar plates, and are usually difficult to distinguish from them; in dividing specimens they often follow a course separate from the plates.

Anterior to the nucleus, the endoplasm of *T. chattoni* contains an abundance of small spherules (fig. A, 5). The zone containing these terminates rather abruptly at the posterior end of the region of thick ectoplasm, near the anterior end of the nucleus. Dubosq and Grassé designated these spherules as anterior lipoid granules; and suggested the possibility of their derivation from the parabasal bodies.

*Trichonympha chattoni* has considerable resemblance to two species that were described earlier: *T. zeylanica* and *T. sphaerica*. The differences from *T. sphaerica* are the more marked. They are considerable in the dimensions of the rostrum, whose length is 12–14 $\mu$ , and whose diameter at the base of the collar is 17–21 $\mu$ , in fixed material of *T. sphaerica*; as compared to a length of 8–9 $\mu$  and a diameter of about 12 $\mu$  in *T. chattoni* from the type host. The nucleus is much larger in *T. sphaerica*, 17–23 $\mu$  as compared to 10–15 $\mu$ ; and it has no evident nucleolus, whereas the nucleolus is a conspicuous structure in *T. chattoni*. The parabasal cords of *T. sphaerica*, although often sinuous, have never been observed to be spiraled as in much material of *T. chattoni*. *T. chattoni* and *T. zeylanica* are compared in the account of the latter species (p. 201).

### *Trichonympha divexa* sp. nov.

(Fig. A, 7)

*Type host*.—*Kalotermes* (s.l.) sp. nov. South Africa.

(Probably a new genus, according to Emerson *in litt.*)

T-4043. Port Shepstone, Natal. (Syntype slides TP-2085:15, 21.)

T-4051. Flagstaff, Cape Province. (Xenosyntype slide TP-2090:9.)

T-4072. Pirie Dam, Cape Province. (Xenosyntype slide TP-2095:5.)

*Diagnosis*.—Length 85–108 $\mu$ , width 43–74 $\mu$ ; dimensions of rostrum: length in center 8–11 $\mu$ , diameter at base of cap 7–8 $\mu$ , diameter at base of collar 12–13 $\mu$ ; rostral tube widened posteriorly; relative length of flagellated area similar to that of *T. chattoni*; parabasal apparatus similar to that of *T. chattoni*, cords sinuous; position of nucleus similar to that in *T. chattoni*; diameter of nucleus 10–14 $\mu$ ; prenuclear endoplasmic granules in small area just posterior to rostral tube; postnuclear endoplasmic spherules present in some specimens.

The species is very much like *Trichonympha chattoni*, and would be identified with it except for a distinct and constant difference in the shape of the rostral tube. In *T. chattoni* the tube is more or less parallel-sided, its posterior diameter being little if any greater than the anterior (fig. A, 4). There is very little variation in this characteristic in different individuals of *T. chattoni* in the type host; and the shape of the tube in the species in all its hosts is the same. In *T. divexa* the rostral tube is much wider at the posterior end than near the anterior end, and is a long cone rather than a parallel-sided tube (fig. A, 7).

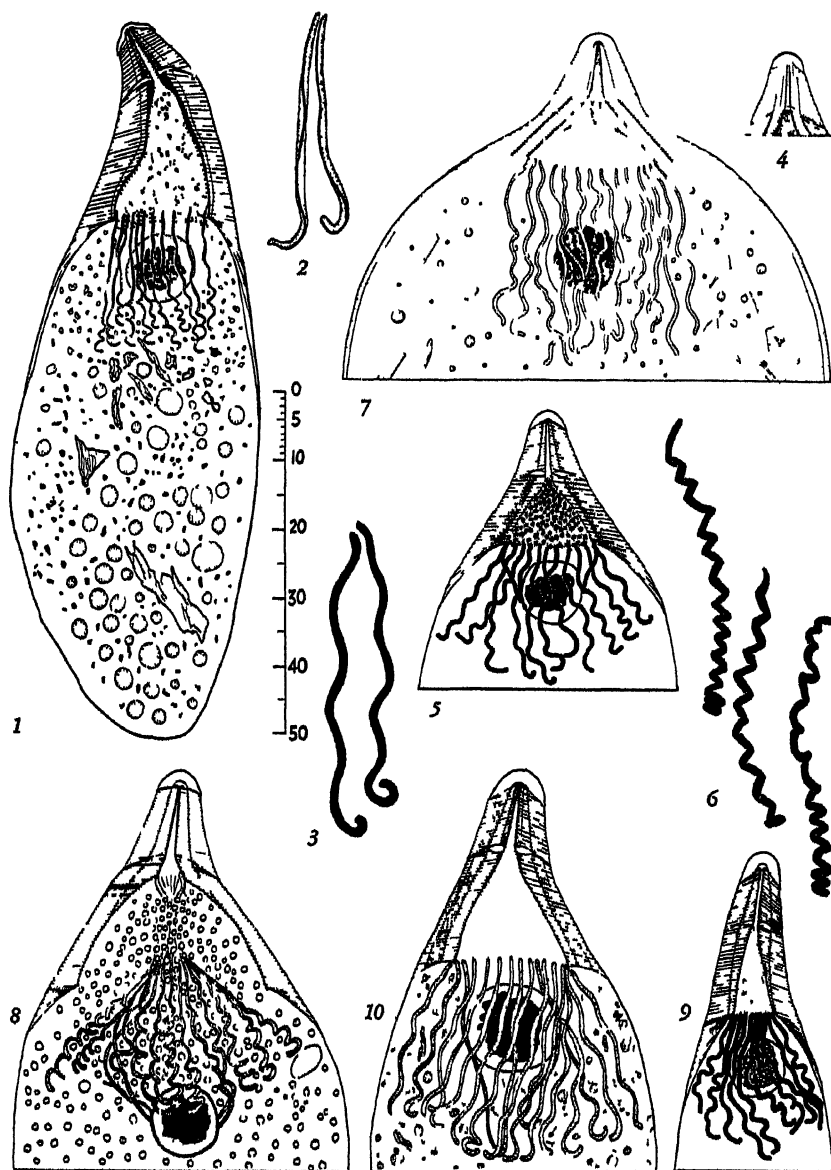


Fig. A. Species of *Trichonympha*. 1-6. *T. chattoni* Duboseq and Grassé. 1. From *Glyptotermes montanus*, Java. Combined from C.R. and S.D. 2-5. From *G. iridipennis*, Australia. 2. Parabasal bodies showing parabasal filament along one margin. F.R. 3. Parabasal bodies solid in appearance, loosely spiraled. F.R. 4. Rostrum, rostral tube not much widened posteriorly. 5. Anterior part of body, coiled parabasals. F.R. 6. From *Glyptotermes neotuberculatus*, Australia; closely spiraled parabasal bodies. 7. *T. devcra* sp. nov. from *Kaloterme* (s.l. now genus?) sp. nov., South Africa; characteristic conical rostral tube. Combined from S.H. and F.R. 8. *T. subquasilla* Kirby from *Kaloterme unigraus*, Galápagos. B.H. 9. *T. tabogae* Kirby from *Kaloterme tabogae*, Panama. Combined from S.D. preparation and restained in protein silver. 10. *T. zeylanica* (Dobell) from *Neoterme militaris*, Ceylon. Combined from S.H. and C.R. 1,  $\times 680$ ; 2, 3, 6,  $\times 1750$ ; others and scale,  $\times 860$ .

**Trichonympha zeylanica (Dobell)**

(Fig. A, 10)

*Gymnonympha zeylanica* Dobell, 1910, *Spolia Zeylanica*, 7:80, pl. 2, fig. 1.*Trichonympha zeylanica* (Dobell). Duboseq and Grassé, 1927, *Arch. Zool. exp. gén.*, 66:465.Kirby, 1932, *Univ. Calif. Publ. Zool.*, 37:417.*Type host*.—*Neotermes militaris* (Desneux). Ceylon.

T-303. (Cleveland-Collier.) (Xenosyntype slides TP-252:4, 11, 18, 19.)

*Additional host*.—*Kalotermes obscurus* (Walker). Australia.

T-520. (Hill 3484, 3485.) S.W. Australia. (Homosyntype slides TP-520:2, 14.)

*Diagnosis*.—(From *Neotermes militaris*, original): length 113 (95–145) $\mu$ ; width 60 (40–75) $\mu$ ; dimensions of rostrum: length in center 11–12 $\mu$ , diameter at base of cap 7–9 $\mu$ , diameter at base of collar 14–17 $\mu$ ; length of flagellated area 28 (25–30) $\mu$ ; ratio of flagellated to non-flagellated area, 0.26:1.00 to 0.43:1.00, averaging 0.32:1.00; parabasal apparatus of sinuous cords, most free from contact with the nuclear membrane, but some in contact; position of nucleus similar to that in *T. chattoni*; chromatin mass a compact ball of strands, diameter of nucleus 12  $\times$  14 (8  $\times$  10—14  $\times$  16) $\mu$ ; neither prenuclear endoplasmic bodies nor postnuclear endoplasmic spherules observed. (From *Kalotermes obscurus*): length 84–160 $\mu$ ; width 44–75 $\mu$ ; dimensions of rostrum: length in center 10.5–12 $\mu$ , diameter at base of cap 6.5–9.5 $\mu$ , diameter at base of collar 12–16 $\mu$ ; length of flagellated area 24–60 $\mu$ ; ratio of flagellated to non-flagellated area 0.40:1.00 to 0.65:1.00; diameter of nucleus 12.1  $\times$  12.7 (10.5  $\times$  10.5–16.0  $\times$  16.0) $\mu$ .

When I examined alcoholic specimens of *Neotermes militaris* in 1932 I was able to recognize as *Trichonympha* the hypermastigote that was present, but I was unable to describe its characteristics in detail. Through the kindness of Professor Cleveland it is now possible to give, from stained smears, a more adequate account of the species.

Dobell (1910) observed the shape of the animal, the flagellar plates, some of the flagella, and the nucleus. The rostrum was not normal in the specimen represented. The flagella were shown only around the base of the rostrum, but actually they arise throughout the length of the flagellar plates. Dobell noted the relatively short extent of the striated area (flagellar plates) and the anterior position of the nucleus, which he found has a diameter of about 15 $\mu$ .

Dobell stated that associated with the larger forms he always found smaller animals, 30–40 $\mu$  in length, possessing a somewhat different structure. He thought these probably represented young stages in the life history of *Gymnonympha*, but he found no very definite intermediate forms. In the small forms, he stated, the nucleus was situated posteriorly. I have examined smears of the flagellates from *Neotermes militaris* in order to determine what Dobell's young form really was, and have had no difficulty in identifying it. It clearly is the large devescovinid present in this termite. At the time Dobell's paper was published, almost nothing was known of devescovinids. Foà's account of *Devescovina striata* had been published, but that gave little information about the organism. Dobell figured the flagellate, and the figure can easily be interpreted as representing the devescovinid. The position, shape, and oblique direction of the longer axis of the nucleus correspond to the situation in the devescovinid; but the nucleus is at the anterior, not the posterior end. The filaments at the other end, which Dobell thought to be flagella, are really adherent microorganisms. That he came close to recognizing that fact is indicated in his paper on the morphology of spirochaetes (1912). In the account of *Treponema termitis* he stated that the organisms have a tendency to attach themselves by one end to the surface of *Gymnonympha*, seeming to form a ciliary investment of the flagellate; and he noted that they may break away and reveal their true nature. He did not state whether he had reference to the larger forms of the flagellate (*Tricho-*

*nympha*) or the so-called small forms (the devescovinid). But adherence of spirochaetes to *Trichonympha* is rare; whereas adherence to devescovinids is frequent, and is, in fact, universal in this large species in *Neotermes militaris*.

*Trichonympha zeylanica* is similar to *T. sphaerica* in the distribution and form of the parabasal cords, the extent of the flagellated area, and the position of the nucleus (fig. A, 10). The parabasals are sinuous or broadly spiraled; they are not so closely spiraled as in much material of *T. chattoni*. Similar parabasal cords occur in some specimens of *T. chattoni*, however. In *T. zeylanica*, as well as in the other two species, some of the centrally located cords come in contact with the nuclear membrane.

In all the material studied, the chromatin mass had contracted some distance away from the nuclear membrane. It filled up more of the interior of the nucleus in the specimen figured by Dobell. The chromatin mass is a dense, compact ball of threads, not separate granules as indicated by Dobell; it is similar to the chromatin mass in the considerably larger nucleus of *T. sphaerica*. In size the nucleus is intermediate between that of *T. chattoni* and *T. sphaerica*.

The prenuclear endoplasm is finely and densely granular, but no deep-staining bodies like the "anterior lipoid granules" of *T. chattoni* were observed in it. In the postnuclear endoplasm the spherules characteristic of *T. chattoni* and *T. sphaerica* were absent from the specimens studied. These endoplasmic spherules were not mentioned or figured by Dobell.

If *T. chattoni* should be shown to be identical with *T. zeylanica* it would be necessary to use the latter local geographical name for that common and very widely distributed species. I believe, however, that there are differences warranting specific distinction. The nucleus is definitely larger. Its size range overlaps that of nuclei of *T. zeylanica*, but whereas the larger sizes are exceptional in *T. chattoni*, and then often enlargement is in preparation for division, the smaller sizes are infrequent in *T. zeylanica*. The parabasal cords, though more or less sinuous, have not been seen so closely spiraled as those of *T. chattoni* frequently are. A usual but variable difference is the greater diameter of the body of *T. zeylanica* at the level of the circular fissure. The minimum of this dimension generally is  $12\mu$  in *T. zeylanica*, whereas in *T. chattoni* the average diameter does not exceed  $12\mu$ .

The characteristics observed in the flagellate from the Australian termite strengthened my conviction of the specific distinction between *T. zeylanica* and *T. chattoni*. The specimens from *K. obscurus* showed similarly the straighter parabasal cords, the greater diameter of the rostrum, particularly at the base of the cap, and the greater diameter of the nucleus. The chromatin mass was a looser ball of threads in *T. zeylanica* from Australia than in the material from the type host, and a nucleolus like that of *T. chattoni* was distinct; the nuclei had been better fixed and the chromatin mass was not contracted. The prenuclear endoplasm in the Australian material was crowded, usually throughout, with small granules. In the postnuclear endoplasm there were often spherules like those of *T. chattoni*, but they were not present in all specimens.

### ***Trichonympha tabogae* Kirby**

(Fig. A, 9)

*Trichonympha tabogae* Kirby, 1932, Univ. Calif. Publ. Zool., 37:398, 446, pl. 80, fig. 64.

Type host.—*Kaloterms tabogae* Snyder. Panama.

T-227. Taboga Island. (Syntype slides TP-136:5, 14, 17, 21.)

Diagnosis.—Length 93 (76–120) $\mu$ ; width 34 (20–50) $\mu$ ; dimensions of rostrum: length in center 9–10 $\mu$ , diameter at base of cap 5 $\mu$ , diameter at base of collar 7–10 $\mu$ ; length of flagellated region 22 (20–25) $\mu$ ; ratio of flagellated to non-flagellated region 0.24:1.00 to 0.38:1.00, averaging 0.30:

1:00; parabasal cords often fairly closely spiraled and arranged as in *T. chattoni*, few or none coming in contact with the nucleus; position of nucleus similar to that in *T. chattoni*; chromatin in small vesicles arranged in linear series or groups, nucleolus of uncertain occurrence, nucleus often pyriform in shape, diameter  $7 \times 9$  ( $6-9 \times 8-10$ )  $\mu$ ; deep-staining granules in prenuclear endoplasm as in *T. chattoni*; spherules in postnuclear endoplasm, small in material studied.

As I noted in 1932, the species is very close to *T. chattoni*, and I have recently reexamined it to determine whether it is not possible to place it in synonymy. It appears, however, best to retain it as a separate species.

In the size, shape, ratio of flagellated and non-flagellated areas, the deep-staining granules in the prenuclear endoplasm, and the parabasal apparatus, *T. tabogae* is similar to *T. chattoni*. The parabasal cords, which have been seen more clearly than when the previous account was prepared, were all spiraled fairly closely and arranged in the same way as in the other species. The rostrum is somewhat more elongated and narrower at the base than in *T. chattoni*.

It is mainly in the nucleus that the characteristics that seem to necessitate its separation exist. The previous account of the arrangement of chromatin in vesicles disposed in linear series or groups has been checked and compared with the structure of the nucleus of *T. chattoni* from the type host. The differences designated then appear to be entirely valid. The chromatin mass does not, of course, appear the same in all preparations; but the differences are apparent in comparable preparations of both species. The nucleolus, supposed then to exist as a small body in *T. tabogae*, is of uncertain occurrence. Another difference, which seems significant, is in the shape of the nucleus. In many specimens it is, as is usual in the genus, spheroidal; but in many others it is more or less pyriform, the smaller end directed anteriorly. In this nuclear shape there is similarity to *Trichonympha grandis* and certain other species from *Cryptocercus punctulatus* (Cleveland *et al.*, 1934). Search of preparations of *T. chattoni* disclosed no instance of similarly shaped nuclei.

### *Trichonympha subquasilla* Kirby emend.

(Fig. A, 8)

*Trichonympha subquasilla* Kirby, 1932, Univ. Calif. Publ. Zool. 27:400, 446, pl. 28, fig. 41; pl. 30, fig. 61.

*Type host*.—*Kaloterme cleavelandi* Snyder. Panama.

T-235. Ancon. (Syntype slides TP-166:6, 150:4.)

*Additional host*.—

*Kaloterme immigrans* Snyder.

T-345. (Light 1293 Ga.) Chatham Island, Galápagos Archipelago. (Homosyntype slides TP-298:2, 8.)

T-4613. (Von Hagen 22.) Hac de Tenguel, Ecuador. (Homosyntype slide TP-3278:4.)

*Diagnosis*.—(From *Kaloterme immigrans* T-345): length 127 (105–145)  $\mu$ ; width 59 (40–77)  $\mu$ ; dimensions of rostrum: length in center 10–12  $\mu$ , diameter at base of cap 7–9  $\mu$ , diameter at base of collar 11–14  $\mu$ ; length of flagellated region 36 (25–50)  $\mu$ ; ratio of flagellated to non-flagellated region 0.32:1.00 to 0.53:1.00, averaging 0.41:1.00; parabasal cords spiraled, as in *T. chattoni*, diverging from a meeting point in the central endoplasm anterior to the nucleus to extend posteriorly, some coming into contact with nucleus, others free; nucleus with a dense, filamentous chromatin mass and a moderate-sized nucleolus, diameter  $9 \times 10.5$  ( $7 \times 9-9 \times 13$ )  $\mu$ ; small deep-staining granules in prenuclear endoplasm; spherules like those of *T. chattoni* in postnuclear endoplasm.

*Trichonympha subquasilla* was not present in a colony of *Kaloterme immigrans* which I obtained at Fanning Island in 1924, nor on a few smears made by Dr. A. M. Adamson from the same species at Oahu in the Hawaiian Islands. The hypermastigote was found, however, on all slides made by Dr. A. E. Larsen from eight colonies of *Kaloterme immigrans* at Narborough, James, Jervis, Indefatigable, South Sey-

mour, Tower, and Chatham Islands in the Galápagos Archipelago. The Galápagos material, from which I reported the species in 1932, was *Kaloterme immigrans*. *Trichonympha* was also present on smears made from *Kaloterme immigrans* in Ecuador by Mr. Wolfgang von Hagen; although the preparations were not good enough to show all the systematic characters well, there is little doubt that the species is *T. subquasilla*.

The additional material from *Kaloterme immigrans* has made possible a more adequate study of this species. There is less relationship to *T. quasilla* than I supposed in 1932. The flagellated region of the body is longer than in that species, and the diameter of the body at the posterior end of the region of thick ectoplasm is greater. The nucleus is situated at or within the posterior limits of the flagellated region.

In form and arrangement the parabasal apparatus is very different from that of *T. quasilla*. In *T. subquasilla* the cords are markedly spiraled, like those characteristic of *T. chattoni*; whereas in *T. quasilla* they extend smoothly, without kinks. The parabasals of the former species are arranged in an unusual manner. This has been observed in many specimens both from *K. immigrans* and *K. clevelandi*. They come together in the central region of the endoplasm in a place often about a third of the distance from the base of the rostrum to the nucleus (fig. A, 8). From this meeting point posteriorly they diverge, describing a cone with more or less convex sides, or sometimes even a bowl form, and extend into the endoplasm of the nuclear region. Some of the parabasals of the interior of the group meet the nuclear membrane in its anterior part or laterally; others have no contact with the nucleus.

From the place where the anterior ends of the stout parts of the parabasal bodies come together the parabasal filaments pass anteriorly in a group. They enter the peripheral endoplasm a short distance posterior to the circular fissure (fig. A, 8).

The nucleus contains a moderate-sized nucleolus, similar to that of *T. quasilla*, and a filamentous chromatin mass that is somewhat denser and more continuous than that of *T. quasilla*.

*Trichonympha subquasilla* is similar in some respects to *T. chattoni*. In the latter species, however, the flagellated region is shorter than that of *T. subquasilla*; and the nucleus is usually closer to the base of the rostrum. The diameter of the body across the posterior ends of the thickened ectoplasm is generally less in *T. chattoni*. The parabasal bodies of that species do not come together in the prenuclear endoplasm at the anterior ends of the stouter part, but extend separately and freely, most of them through the peripheral endoplasm, continuing in filaments that pass separately to the region of the rostrum.

### *Trichonympha lighti* Kirby

(Fig. B, 2)

*Trichonympha lighti* Kirby, 1932, Univ. Calif. Publ. Zool., 37:401, 447, pl. 27, figs. 38-40; pl. 30, fig. 56.

Type host.—*Kaloterme emersoni* Rowan. Mexico.

T-262. (Light 113.) Colima. (Syntype slides TP-224:22, 27, 29, 37.)

**Diagnosis.**—Length 113 (94-188) $\mu$ ; width 52 (36-65) $\mu$ ; dimensions of rostrum: length in center 11-12 $\mu$ , diameter at base of cap 6-8 $\mu$ , diameter at base of collar 11-13 $\mu$ ; length of flagellated region about 30-50 $\mu$ ; ratio of flagellated to non-flagellated region 0.36:1.00 to 0.50:1.00 averaging 0.42:1.00; ectoplasm of flagellated region of uniform thickness, with rather abrupt termination; parabasal apparatus of 30-40 slender cords, sinuous but not closely spiraled, diverging from a meeting place anterior to the nucleus, most coming in contact at their ends only with the anterior or lateral nuclear membrane, some ending freely; distance from anterior end of body to anterior end of nucleus 29 (11-42) $\mu$ ; diameter of nucleus 8 (6.5-9.5) $\mu$ ; numerous small, deep-staining granules in prenuclear endoplasm; spherules in postnuclear endoplasm.



The rostral tube is almost parallel-sided, with an abrupt diminution in diameter shortly before it meets the crescentic bodies. I have seen no central rod in the anterior part of the rostral tube.

In the arrangement of the parabasal cords (fig. B, 2) *T. lighti* is closest to *T. subquasilla*. The cords appear first in the posterior part of the flagellated region, where their anterior ends are gathered more or less into a central group. The parabasal filaments have not been seen in *T. lighti*, but they probably extend, as in *T. subquasilla*, in a central column from the base of the rostral tube. From their junction with the filaments the cords extend posteriorly and laterally. Most of them ultimately turn in toward the nucleus, coming in contact at the extreme end with the anterior or lateral parts of the nuclear membrane. Some cords may end freely. The group of parabasal cords is often outlined in a more or less fusiform figure, with the nucleus attached at one end, and half the nucleus reaching free beyond it. The thickness of the spindle varies greatly, from about the diameter of the nucleus to an almost spherical form. In some specimens the terminal parts of the parabasal cords reach around the nucleus or even extend a short distance posterior to it; but usually only the anterior part of the nucleus is in contact, and that with the very ends of the cords.

There are distinct differences in the parabasal apparatus of *T. lighti* and *T. subquasilla*. The cords of the former species are broadly sinuous, and I have never observed them to be closely spiraled. The latter form is characteristic of the other species. There is a much more marked tendency toward contact of the cords with the nucleus in *T. lighti* than in *T. subquasilla*.

Another marked difference is in the conspicuously smaller nucleus of *T. lighti*. The mean and range in diameter given in the diagnosis are based on fifty nuclei. Many more were observed to ascertain that the minimum and maximum were not exceeded; and similar results were obtained in the previous (1932) measurements of nuclei.

In most specimens of *Trichonympha lighti* there is present in the cytoplasm an ellipsoidal or spheroidal body. It occupies any possible position in the postnuclear endoplasm, and I have never seen more than one. It varies greatly in size, from much smaller to considerably larger than the nucleus. The inclusion consists of a dark, heterogeneous mass surrounded by a membrane, with a narrow clear space between it and the membrane. In most preparations its stainability with haematoxylin is much less than that of the chromatin. In certain preparations there is a coarse striation in the direction of the longer diameter, the striae staining deeply and being distributed throughout the substance of the dark body. There is a superficial resemblance to a dividing ciliate micronucleus. This structure recalls the inclusion reported by Dunkerly (1923) in *Pseudotriconympha sphaerophora*.

### ***Trichonympha saepicula* Kirby emend.**

(Fig. B, 1)

*Trichonympha saepiculae* Kirby, 1932. Univ. Calif. Publ. Zoöl., 37:403, 447, pl. 25, figs. 20-30; pl. 26, figs. 32-34; pl. 30, figs. 62-63; fig. C.

*Type host*.—*Rugitermes kirbyi* Snyder. Costa Rica. (Locality incorrectly given as Panama in 1932.)

T-148. Cartago. (Syntype slides TP-92:3, 5, 17.)

T-135. Cartago. (Xenosyntype slide TP-76:15.)

*Additional host*.—

*Rugitermes panamae* Snyder. Panama.

T-120. Barro Colorado. (Homosyntype slides TP-58:3, 5; TP-56:19.)

*Diagnosis*.—Length 112 (72-149) $\mu$ ; width 48 (29-66) $\mu$ ; dimensions of rostrum: length in center 11-13 $\mu$ , diameter at base of cap 6-8 $\mu$ , diameter at base of collar 11-13 $\mu$ ; ratio of flagellated

to non-flagellated region 0.37:1.00 to 0.75:1.00, averaging 0.55:1.00; parabasal apparatus of about 40 slender cords beginning in the peripheral endoplasm in the posterior part of the flagellated region, forming a single-layered cylindrical case around the nucleus, all usually adhering to nuclear membrane, continuing posterior to nucleus for a variable distance; distance from anterior end of body to anterior end of nucleus 18–32 (44)  $\mu$ ; diameter of nucleus 13 (10–17)  $\mu$ ; chromatin in irregular, coiled, varicose strands, small nucleolus; prenuclear endoplasmic granules abundant, small, massed; postnuclear endoplasmic inclusions; numerous spherules as in *T. chattoni*.

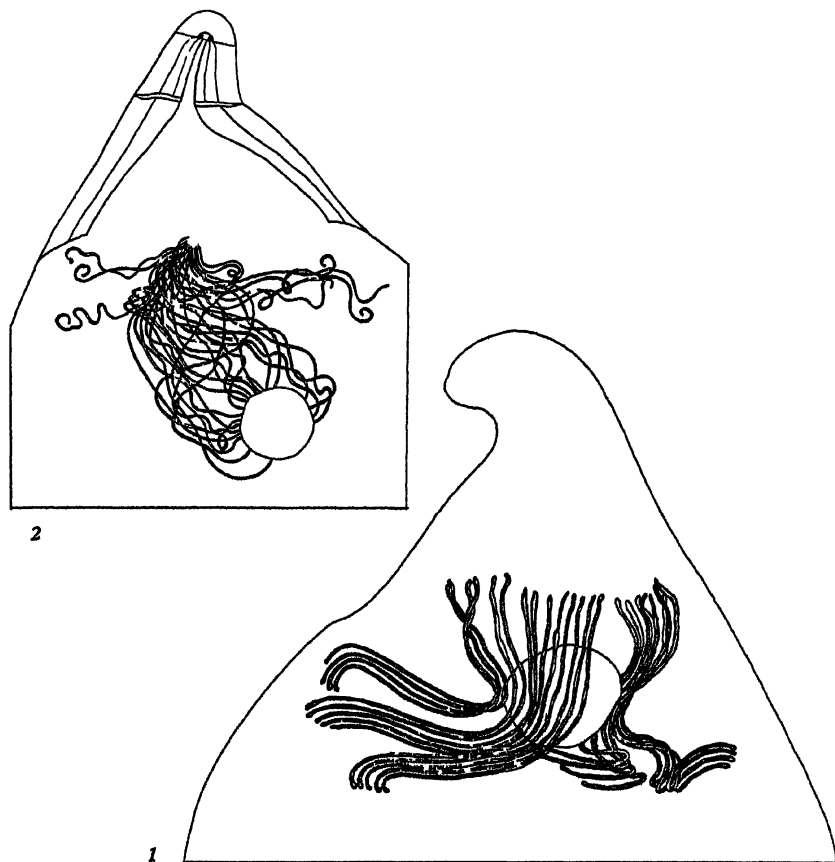


Fig. B. Parabasal apparatus.  $\times 1275$ . 1. *Trichonympha saepicula* Kirby from *Kaloterms panamae*, Panama. 2. *Trichonympha lighti* Kirby from *Kaloterms emersoni*, Mexico.

*Trichonympha saepicula* resembles members of the *T. chattoni* group in most respects, but differs markedly from the other species in the parabasal apparatus. The rostral tube is shaped like that of *T. chattoni*. The anterior ends of the parabasal cords are at one level in the peripheral endoplasm, usually some distance anterior to the posterior ends of the flagellar plates. For the most part, the cords come in contact with the sides of the nuclear membrane, and may extend along it, but they do not round the posterior end of the nucleus. The cage-like structure that they form (fig. B, 1) was described in my previous account of the species (1932, p. 403). In the parts that extend posterior to the nucleus the cords are often arranged in flat groups, generally of three to six. Their arrangement is comparable to that in *T. collaris*. The position of the nucleus varies to some extent, and rarely is it so far posterior that the ends of the cords adhere to the membrane. In many specimens, on

the other hand, the length of the part of the cords posterior to the nucleus exceeds that anterior to the nucleus. The cords are connected by filaments to the region at the base of the rostral tube.

***Trichonympha ampla* sp. nov.**

(Fig. C, 1-5)

*Type host*.—*Kaloterмес occidentis* Walker. Mexico.

T-356. (Light.) Lower California. (Syntype slides TP 317:25, 28.)

*Diagnosis*.—Length 172 (94-287) $\mu$ ; width 72 (41-126) $\mu$ ; dimensions of rostrum; length in center 11-13 $\mu$ , diameter at base of cap 8-10 $\mu$ , diameter at base of collar 15-21 $\mu$ ; length of flagellated region 85 (31-150) $\mu$ ; ratio of flagellated to non-flagellated region 0.44:1.00 to 1.67:1.00, averaging 1.01:1.00; number of flagellar plates in body region in the neighborhood of 60; parabasal cords long, leaving periphery of endoplasm a short distance posterior to the circular fissure, sometimes distributed separately in endoplasm, characteristically forming a central columnar apparatus within the posterior part of which the nucleus is situated; distance from anterior end of body to anterior end of nucleus 28 to 86 $\mu$ ; nucleus spheroidal or ellipsoidal, diameter 13 (8-17) $\mu$ ; chromatin in reticular group of coarse, varicose strands, spherical peripheral nucleolus; spherules, like those of *T. chattoni*, abundant in postnuclear endoplasm.

Unfortunately, I have had comparatively little material of this interesting species; the description is necessarily incomplete and preliminary. The species is clearly distinct from any other I have studied, and it is desirable to include in this paper what information can be obtained about it.

It is a large species, the largest so far found in termites of the family Kalotermitidae. It has been seen only in fixed preparations, and in making the measurements recorded above (from 35 specimens) only those that appeared to be of normal shape were included. This shape is somewhat elongated, but there were present also many broadly rounded, almost globular forms. Where these fit into the picture of the species cannot be said until living, or at least better prepared, material is available.

The rostral tube is rather straight-sided, tapers gradually from the posterior to the anterior end, and terminates in two crescentic bodies. In the center of the anterior part of the rostral tube is a straight, slender rod about 4 $\mu$  in length.

Posterior to the circular fissure for a distance often of 15-20 $\mu$  the endoplasm is crowded with the prenuclear granules. The parabasals appear first in the peripheral endoplasm at the posterior end of this region. Parabasal filaments continue anteriorly to the base of the rostral tube; it was difficult to see them. In the arrangement that is most characteristic of *T. ampla* the parabasals, passing posteriorly, organize themselves in an even, cylindrical layer, generally spiraled somewhat in a laeotropic direction. They thus form a more or less columnar structure extending centrally in the endoplasm and in its posterior part surrounding the nucleus. The cords do not become evenly arranged until a certain distance after they leave the peripheral endoplasm, and in this region there may be a more or less deep constriction in the figure. The diameter of the figure in its middle part is often about 1½ to 2 times the diameter of the nucleus. Many of the parabasals, sometimes all of them, come in contact with the nuclear membrane at the sides. They continue for a short distance posterior to the nucleus, and here they become very irregular in arrangement.

Although the development of such a columnar parabasal apparatus is characteristic of the species, it does not occur in all individuals. It is present especially in the more elongated specimens, in which the flagellated region is relatively long. There are many specimens in which the parabasal cords are arranged more like those of *T. chattoni*. They run separately in various parts of the endoplasm, and

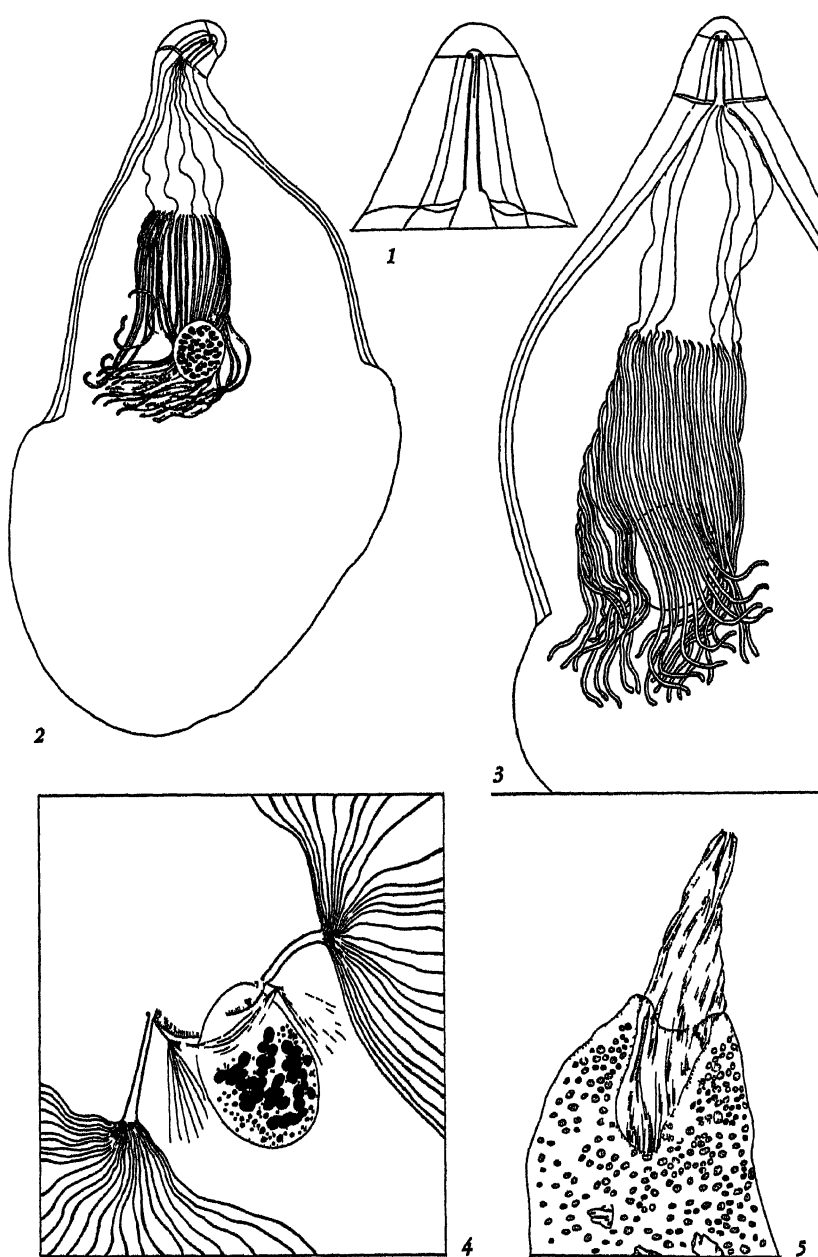


Fig. C. *Trichonympha ampla* sp. nov. from *Kaloterms occidentis*, Mexico. 1. Parabasal apparatus shown only in lower half, upper part over nucleus omitted.  $\times 525$ . 2. Rostrum, showing rostral tube, optical section of crescentic body at anterior end of each half of rostral tube, central rod in anterior part of tube.  $\times 1700$ . 3. Parabasal apparatus, cords extended in filaments to base of rostral tube. 4. Prophase, showing extranuclear spindle, astral rays, new rostral tube halves, short rod from each pole of the spindle to the region of the anterior end of the new rostral tube half.  $\times 1275$ . 5. Posterior end of *Trichonympha* with large fragment of wood partly ingested. Spherules in cytoplasm.  $\times 525$ .

are not gathered centrally. There are various dispositions between this one of dispersed parabasal cords and the columnar figure into which all the cords are incorporated.

In the presence of abundant spherules in the endoplasm of the non-flagellated region, *T. ampla* is similar to *T. chula*, *T. parva*, *T. sphaerica*, and probably all known species of *Trichonympha* in the Kalotermitidae.

### *Trichonympha quasilla* Kirby emend.

(Fig. D, 2, 3)

*Trichonympha quasilla* Kirby, 1932, Univ. Calif. Publ. Zoöl., 37:399, 446, pl. 28, fig. 42; pl. 30, figs. 57-58.

*Type host*.—*Kalotermes perezii* Holmgren, Costa Rica. (Determined by A. E. Emerson; incorrectly recorded by Kirby, 1932, as *K. snyderi*.)

T-141. Cartago. (Syntype slide TP-88:11.)

*Diagnosis*.—(Revised.) Length 97 (75-128) $\mu$ ; width 40 (25-58) $\mu$  dimensions of rostrum: length in center 8-11 $\mu$ , diameter at base of cap 5-7 $\mu$ , diameter at base of collar 9-13 $\mu$ ; length of flagellated region 21 (15-25) $\mu$ ; ratio of flagellated to non-flagellated region 0.21:1.00 to 0.37:1.00, averaging 0.28:1.00; ectoplasm of flagella-bearing region with a uniform thickness of about 4 $\mu$ , with abrupt termination, diameter across posterior end of this zone of ectoplasm 16-28 $\mu$ ; parabasal cords forming an elongated, pouch-shaped structure in the bottom of which the nucleus is located; anterior end of nucleus 27-41 $\mu$  from anterior end of body, thus being some distance posterior to the flagellated region, measuring  $7 \times 8-10 \times 12\mu$ , chromatin in isolated granules or more frequently in loosely arranged strands, with moderate-sized interiorly placed nucleolus; endoplasm of flagellated region densely and finely granular, endoplasm of non-flagellated region containing numerous spherules.

The parabasal apparatus is the most distinctive feature of this species. The cords are all applied in their terminal parts to the nucleus, around which they bend; they have not been observed to extend free beyond the nucleus (fig. D, 3). This arrangement gives a corbule as in *T. agilis* and *T. corbula*, and it has a characteristic shape. The body is relatively narrow across the posterior end of the thickened ectoplasm of the flagellated region, and the nucleus is often 10-15 $\mu$  posterior to that region. The cords, in consequence, form an elongated pouch instead of a more or less shallow bowl; and in most specimens the widest part of the pouch is not at its anterior part, but near or posterior to the middle. The cords are smooth in outline and evenly curved, and the space between adjacent cords is generally narrower than the breadth of the cords.

In the endoplasm enclosed by the pouchlike parabasal apparatus there is a central group of filaments, sinuous in their course and stout for filaments, but considerably more slender than the above described parabasal cords (fig. D, 2). These filaments extend from the anterior endoplasm and generally meet the anterior surface of the nuclear membrane, but sometimes stop short of this; in the form and position of this group there is a suggestion of the nuclear sleeve shown by Cleveland *et al.*, (1934) in species of *Trichonympha* from *Cryptocercus*. But the material from *K. perezii*, though limited in amount and somewhat obscure, showed definitely sinuous fibrils in a solid rather than a tubular distribution. Further discussion of this structure is given below (p. 216).

The chromatin mass in the nucleus of *T. quasilla* is less dense than in many other species of *Trichonympha*. The chromatin is in granules that sometimes are isolated, but more often are grouped in threads, which appear moniliform or solid. These threads vary in length but do not constitute a continuous spireme and they are rather loosely arranged. There is a spherical nucleolus of moderate size, often about 2.5 $\mu$  in diameter. It is near one edge of the chromatin mass, but generally interior to the mass itself.

*Trichonympha corbula* sp. nov.

(Fig. D, 1; pl. 12, figs. 1-5)

*Type host*.—*Procryptotermes* sp. nov. Madagascar.

T-4374. Near Mahabo. (Syntype slides TP-3157:12, 14.)

T-4307. Near Maevetanana. (Homosyntype slide TP-3094:15.)

*Additional hosts*.—*Kaloterms* (s.l.) *castaneiceps* Sjöstedt. Madagascar.

T-4505. Tamatave. (Homosyntype slides TP-3205:13.)

*Kaloterms* (s.l.) *longus* (Holmgren). Madagascar.

T-4460. Manantantely. (Homosyntype slides TP-3181:19, 24.)

T-4421. Near Tongobory. (Homosyntype slide TP-3160:12.)

*Kaloterms* (s.l.) sp. nov. Madagascar.

T-4468. Near Ambovombe. (Homosyntype slide TP-3171:5.)

(According to Emerson, *in litt.*, those three termites belong to a new genus of Kalotermitinae.)

*Diagnosis*.—(From type host): length 118 (90-165) $\mu$ ; width 66 (41-110) $\mu$ ; dimensions of rostrum: length in center 9-11 $\mu$ , diameter at base of cap 5-7 $\mu$ , diameter at base of collar 10-13 $\mu$ ; length of flagellated region 43 (25-67) $\mu$ ; ratio of flagellated to non-flagellated region 0.35:1.00 to 0.75:1.00, average 0.62:1.00; ectoplasm of flagella-bearing region of even thickness; parabasal apparatus of numerous cords all forming a corbule, ends applied to nuclear membrane, usually not extended posterior to it; nucleus located just posterior to the posterior limit of the flagellated zone, often with its anterior end at a level with that limit, diameter 10 (8-13) $\mu$  chromatin in stout, varicose strands, spherical nucleolus often located somewhat interiorly; in prenuclear endoplasm numerous small, deep-staining granules not massed or conspicuous; in postnuclear endoplasm small fragments of wood and spherules averaging about 2 $\mu$ . (From *Kal.* (s.l.) *longus*): length 107 (85-120) $\mu$ ; width 59 (40-70) $\mu$ ; dimensions of rostrum: length in center 8-9 $\mu$ , diameter at base of cap 4-6 $\mu$ , diameter at base of collar 8-10 $\mu$ ; length of flagellated region 34 (25-40) $\mu$ ; ratio of flagellated to non-flagellated region 0.37:1.00 to 0.67:1.00, averaging 0.47:1.00; diameter of nucleus 10  $\times$  10-13  $\times$  15 $\mu$ . (From *Kal.* (s.l.) *castaneiceps*): length 73 (55-90) $\mu$ ; width 48 (20-67) $\mu$ ; dimensions of rostrum: length in center 8-9 $\mu$ , diameter at base of cap 5 $\mu$ ; diameter at base of collar 8-13 $\mu$ ; length of flagellated region 22 (12-30) $\mu$ ; diameter of nucleus 10 (8-13) $\mu$ .

The corbule of *T. corbula* is a well-formed, bowl-shaped structure, into the formation of which all the parabasal bodies enter (fig. D, 1). The cords appear first in the peripheral endoplasm in the posterior half of the flagellated area. Their posterior extremities are usually applied closely to the nuclear membrane; only exceptionally do they extend into the endoplasm posterior to the nucleus. This posterior extension of the parabasal bodies for a short distance beyond the nucleus was observed chiefly in some specimens from *Kaloterms longus*. The cords may be arranged longitudinally and symmetrically; but in preparations they are often more or less sinuous, oblique, and not strictly parallel to one another.

The prenuclear endoplasm contains numerous small, deep-staining granules; these are not conspicuously massed.

In the postnuclear endoplasm there are fragments of wood, which generally are small in size and are not very numerous. Also present are deep-staining granules, like those in prenuclear endoplasm. Many specimens contain numerous spherules, which do not stain but appear brownish in prepared material. These vary in size; many are about 2 $\mu$  in diameter, but others range from a fraction of a micron up to 4 $\mu$ .

Among previously described species, only those that have the parabasal apparatus in the form of a basket, with all cords applied at the posterior ends to the nuclear membrane, need be compared with *T. corbula*. Those species are *T. agilis*, *T. quasilla*, and *T. teres*.

The corbule of *Trichonympha agilis*, in the arrangement of the cords and their relationship to the nucleus, is very much like that of *T. corbula*. The rostrum is more similar in the two species than would appear from the diagnosis of *T. agilis* given

by me (1932). The measurements recorded there were from living material; those of fixed specimens are less, and only they can be compared with those of *T. corbula* recorded here. The dimensions of the rostrum of *T. agilis* in measurements of fixed material made recently were: length in center  $8\mu$ , diameter at base of cap  $5-6\mu$ , diameter at base of collar  $11-12\mu$ . These are similar to the measurements of *T. corbula*. The measurements recorded for that and for most other species of *Trichonympha* are of fixed material; and since it is clear that in many instances only such material will be available for measurements, diagnosis should contain those data, although there appears to be considerable alteration upon fixation. Regarding the matter in another way, the measurements given, particularly of the rostrum, for all species of *Trichonympha* described in this paper are different from the actual size of living specimens.

Not only is the length of *T. corbula* greater, but the body has a much broader form than that of *T. agilis*. The average breadth is about twice as great as in the latter species.

Most nuclei of *T. agilis* measure  $10-13\mu$ , though there are some in fixed material as small as  $8\mu$ . Nuclei of *T. corbula* are about the same size, but there is some difference in structure. The arrangement of chromatin in a compact mass of separate, or partly separate, granules, noted in 1932, is characteristic of *T. agilis*, though there are some reticular chromatin masses. In *T. corbula*, however, the chromatin is in strands, in many instances more loosely arranged than in *T. agilis*.

A notable difference between the two species is the absence in *T. agilis* of the brownish spherules, which are abundant in the postnuclear endoplasm of *T. corbula*.

So far as can be judged from fixed and stained material, the flagella extend considerably farther beyond the posterior end in *T. agilis* than in *T. corbula*.

*Trichonympha quasilla* is similar to *T. corbula* in size, though it is somewhat more slender. The rostrum is comparable in size. The zone of thickened ectoplasm through which the roots of the flagella extend is ordinarily considerably longer in *T. corbula*. The diameter of the body across the posterior end of this thickened zone, at the point where it begins to taper to the end of the flagellated area, is  $35-45\mu$  in *corbula*, compared to  $16-28\mu$  in *quasilla*. These differences in the shape of the prenuclear part of the body constitute a very marked distinction between the two species. There is a conspicuous difference in the shape of the corbule. In *T. quasilla*, the corbule has a pouch form with a lesser diameter at the anterior end than at the widest part (fig. D, 3). In *T. corbula* the typical form of the apparatus is bowl-like (fig. D, 1).

### *Trichonympha teres* sp. nov.

(Fig. D, 4, 5)

*Type host*.—*Neotermes meruensis* Sjöstedt. Tanganyika Territory.

T-2009. Near Arusha. (Syntype slides TP-1089 2, 15.)

T 3036. Near Ngaiongoro Crater. (Xenosyntype slides TP 2046.5, 20.)

*Diagnosis*.—Length  $94 (70-125)\mu$ ; width  $66 (40-100)\mu$ ; dimensions of rostrum: length in center  $9-10\mu$ , diameter at base of cap  $5-6\mu$ , diameter at base of collar  $9-10\mu$ ; length of flagellated area  $41 (25-55)\mu$ ; ratio of flagellated to non-flagellated region  $0.45:1.00$  to  $1.00:1.00$ , averaging  $0.76:1.00$ ; ectoplasm and parabasal apparatus as in *T. corbula*; nucleus located near the posterior limit of the flagellated zone, size  $16 \times 17 (12 \times 15-16 \times 20)\mu$ , chromatin mass a dense grouping of coiled threads, minute stainable granules in prenuclear endoplasm, brownish spherules in post-nuclear endoplasm.

*Trichonympha* was absent from most colonies of *Neotermes meruensis*. Twelve colonies in all were obtained, three from Taveta Forest, Kenya Colony, six from the region around Mount Kenya, and one from near Mbeya, Tanganyika, in addition to the two recorded above as hosts of the hypermastigotes. In ten of the twelve colonies no *Trichonympha* was found.

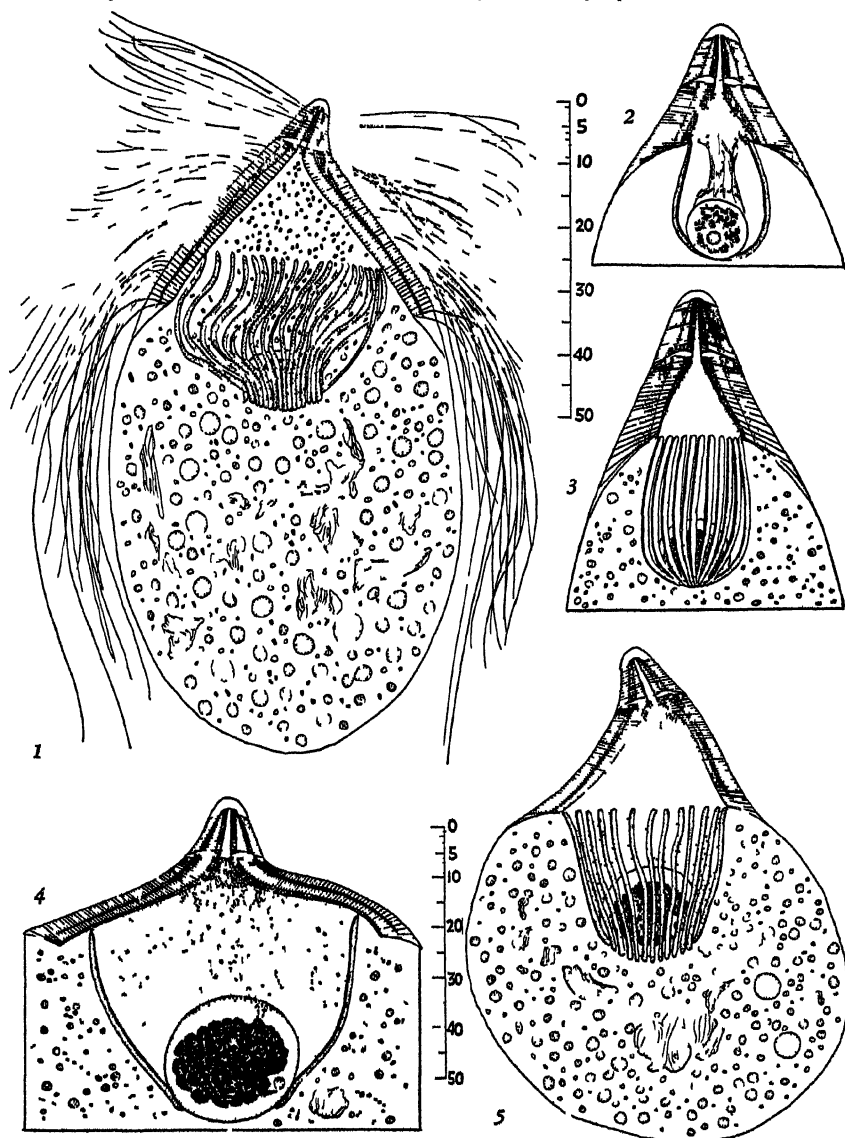


Fig. D. Species of *Trichonympha* 1. *T. corbula* sp. nov. from *Picrocyptotermes* sp. nov., Madagascar. Holl. II. 2-3. *T. quasilla* Kubby from *Kalotermea petersi*, Costa Rica. S.H. Er. 2. Optical section showing outermost parabasal cords and central group of filaments. 3. Surface aspect of parabasal apparatus. 4, 5. *T. teres* sp. nov. from *Neotermes mei uensis*, Tanganyika Territory. S.H. 4. Optical section showing outermost parabasal cords. 5. Surface aspect of parabasal apparatus. 1, 5,  $\times 680$ ; others,  $\times 860$ .

*Trichonympha teres* is related to *T. corbula*, but there are certain well-marked differences. The body as a whole appears more rounded than in *T. corbula* (fig. D, 5), and there is usually not so marked an elongation of the parts anterior to the nucleus (fig. D, 4). The most notable difference is the much larger nucleus, the average diameter of which exceeds that of *T. corbula* by more than 50 per cent. The chromatin mass in the nucleus is particularly dense, appearing as a close tangle of threads which generally is intensely stained.



***Trichonympha peplophora* sp. nov.**

(Fig. E, 1-4; fig. I, 4, 5)

*Type host*.—*Neotermes howa* (Wasmann). Madagascar. Mauritius.

T-4446. Manantantely, Madagascar. (Syntype slides TP-3170:10, 17, 18, 33.)

T-4522. Mauritius. (Homosyntype slide TP-3202:53.)

T-4523. Mauritius. (Homosyntype slide TP-3203:16.)

*Additional hosts*.—*Neotermes desneuxi* (Sjöstedt). Madagascar.

T-4452. Nampohana. (Homosyntype slide TP-3177:6; 3188:8.)

*Neotermes gracilidens* (Sjöstedt). Madagascar.

T-4487. Perinet. (Homosyntype slide TP-3185:1.)

T-4489. Perinet. (Homosyntype slide TP-3183:3.)

*Neotermes amplius* (Sjöstedt). Madagascar.

T-4500. Perinet. (Homosyntype slide TP-3268:11, 14.)

*Diagnosis*.—(From type host): length 134 (100–165) $\mu$ ; width 63 (50–110) $\mu$ ; dimensions of rostrum: length in center 9–11 $\mu$ , diameter at base of cap 5–7 $\mu$ , diameter at base of collar 9–12 $\mu$ ; length of flagellated region 84 (45–110) $\mu$ ; ratio of flagellated to non-flagellated region 0.82:1.00 to 3.00:1.00, averaging 1.67:1.00; parabasal cords originating at various levels in the peripheral endoplasm of the posterior half of the flagellated region, some coming in contact with the sides of the nucleus and passing beyond it, others free of contact with the nuclear membrane; chromatin mass compact, of coiled varicose strands, with one or often two spherical nucleoli of moderate size, diameter 13.1 (11–15) $\mu$ ; prenuclear endoplasm in anterior part densely crowded with deep-staining granules and rods; postnuclear endoplasm with abundant spherules, many 2–3 $\mu$  in diameter. (From *Neotermes howa*, Mauritius): length 126 (80–200) $\mu$ ; width 63 (50–120) $\mu$ ; dimensions of rostrum: length in center 9–12 $\mu$ , diameter at base of cap 6–8 $\mu$ , diameter at base of collar 11–16 $\mu$ ; length of flagellated region 57 (35–80) $\mu$ ; ratio of flagellated to non-flagellated region 0.67:1.00 to 2.96:1.00, averaging 1.21:1.00; nuclear size 11.3 (9–16) by 14 (10–17) $\mu$ .

The rostral tube of *Trichonympha peplophora* (fig. E, 2, 4) is shaped somewhat like a slender, long-necked bottle. The anterior part tapers to less than half the diameter of the posterior part; sometimes there is also a slight narrowing just anterior to the posterior end. At the anterior end, at the place where the layers of ectoplasm terminate and give way to the clear region under the operculum, the rostral tube comes in contact with a body that in heavily stained material appears as a hemispherical granule. In better differentiated material, however, it appears that this is not a solid granule. There appear in optical section, when the flagellate is suitably oriented, two granule-like structures, one at the end of each half of the rostral tube (fig. E, 2). The apparent granules are optical sections of elongated, crescentic bodies. I have found no central rod within the anterior part of the rostral tube.

The cordlike parts of the parabasal bodies begin in the peripheral endoplasm at various positions in the posterior half of the prenuclear area, where their anterior ends are close to the layer of basal bodies of the flagella (fig. E, 1). The parabasal cords are circular in cross section, and consist of two elements, namely, a sharply defined filament, which stains black when the rest of the parabasal body is unstained, and which extends along the entire edge of the parabasal body, and a second substance which gives thickness to the cords (fig. E, 3). At the proximal end the filaments continue alone to the region of the base of the rostrum, situated in the peripheral part of the endoplasm.

Those parabasal bodies of which the cordlike part begins more anteriorly constitute a suspensory apparatus for the nucleus (fig. E, 1). They pass inward steeply, and come into contact with the membrane at the sides of the nucleus. After extending along the nuclear membrane for a short distance, they may bend away from the nucleus and continue for some distance in the endoplasm, but sometimes

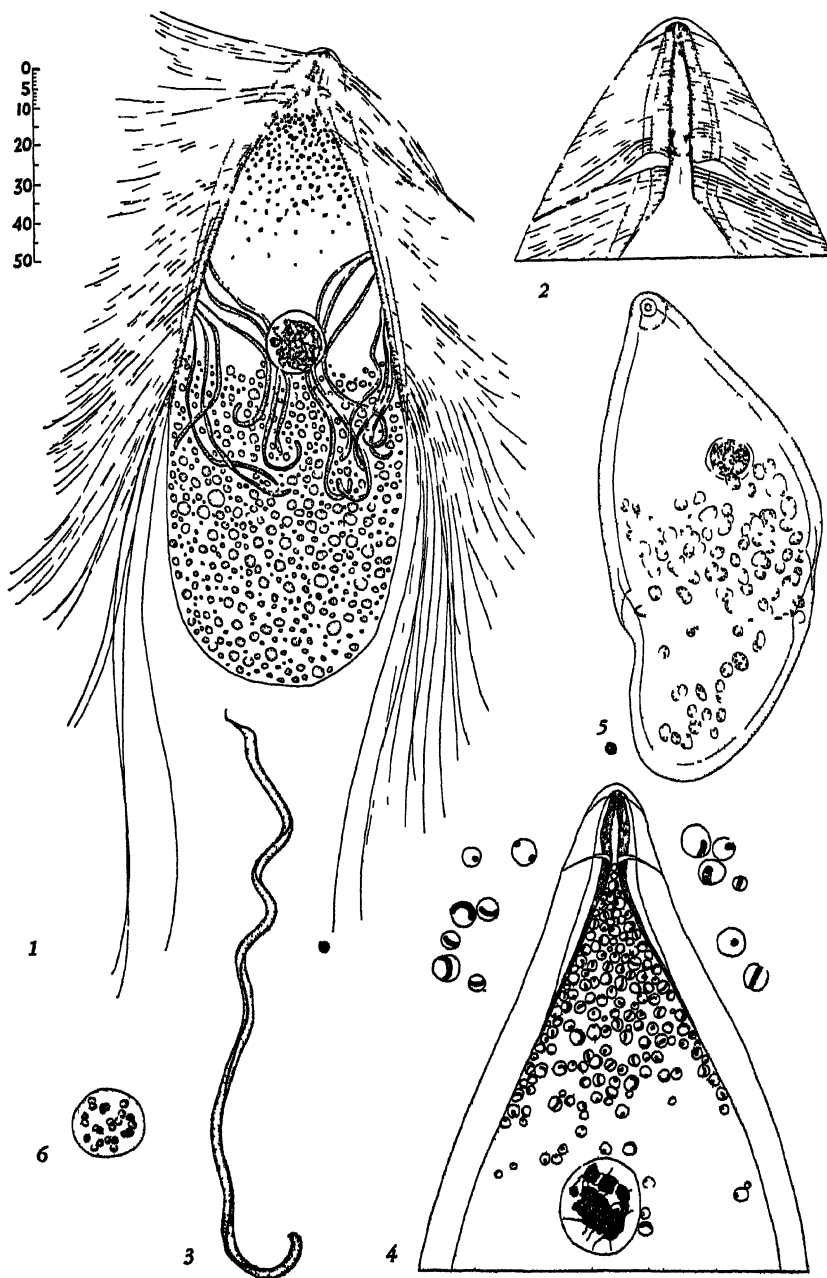


Fig. E. 1-3. *Trichonympha peplophora* sp. nov. from *Neotermes howa*, Madagascar. 1. Optical longitudinal section, with full lengths of flagella at margins. Combined from Holl. H. and S.H.  $\times 510$ . 2. Actual longitudinal section of rostrum, free parts of flagella omitted. S.H.  $\times 1750$ . 3. Parabasal body, showing marginal parabasal filament. From actual longitudinal section. S.H.  $\times 1750$ . 4. *T. peplophora* from *N. howa*, Mauritius. S.H. Actual longitudinal section of anterior part of body,  $\times 860$ . On either side, beside the body, the spherules of the prenuclear endoplasm are shown in greater detail.  $\times 1750$ . 5. *T. turkestanica* Bernstein from *Anacanthotermes ochraceus*, Egypt, containing *Sphaerita*-like parasites in endoplasm. F.R.  $\times 320$ . 6. Detail of one of these parasites.  $\times 1800$ .

they pass more directly posteriad. In the former arrangement, which is characteristic of the species, the nucleus is at the waist of a broad hourglass-shaped grouping of parabasal cords.

There exists another group of parabasal cords, consisting of those that establish no contact with the nuclear membrane. These appear first in the peripheral endoplasm of the posterior part of the flagellated area, at the level of the nucleus, or near it. They pass into the endoplasm of the non-flagellated area, terminating at about the same level as the parabasal cords of the corbule.

The nucleus of *T. peplophora* is usually spheroidal, sometimes ellipsoidal in form. The chromatin mass fills the whole interior or, as is common in fixed material, is contracted a little away from the membrane. No deep-staining granules are present in the clear zone. The chromatin is in a mass of coiled strands, with no limitation to a special abundance of chromatin material at the periphery as compared to the interior of the mass. Smooth strands of approximately even diameter, separated by interstices of considerable size, are found in somewhat enlarged nuclei, which have probably entered upon prophase changes. In most interphase nuclei the strands are definite, but are varicose and uneven in diameter; often they present the appearance of irregular chromatic masses interconnected by bridges and filaments of the same substance. These strands are packed more densely than in prophase nuclei. In nuclei of the smallest size the chromatin is generally aggregated into a sharply circumscribed mass separated from the membrane by a rather broad clear space.

The presence of a nucleolus in the outer part of the chromatin mass is characteristic of the species. In many specimens there are two. They are often about  $2\mu$  in diameter, but may be much smaller, and in some nuclei are enlarged to 4 and even to  $5\mu$ . They are spherical or spheroidal in form.

The anterior part of prenuclear endoplasm is densely packed with bodies that often stain deeply in iron-haematoxylin (fig. E, 1, 4). They are especially numerous in the region just posterior to the rostrum, when the endoplasm is filled with what sometimes appears as an almost solid black mass. In this region the granules are not in direct contact with the layer of basal granules of the flagella, but are separated from it by a clear space. From the mass they extend a variable distance posteriorly. Usually only scattered ones are present posterior to the first third or half of the prenuclear area. In some individuals the bodies all appear as granules; in others, rods are characteristic; in still others, the types are mixed. The bodies vary considerably in size, and many exhibit various irregularities in form. With Delafield's haematoxylin after Schaudinn fixation these bodies remain unstained, but their presence can be detected because of their high refractivity. With Heidenhain's iron-haematoxylin after Schaudinn or Fleming fixation, the bodies may stain deeply in some material and be unstained in flagellates on other slides, or even in other specimens on the same slide. Sometimes some are stained and others unstained in the same specimen.

In whole mounts the bodies usually, whether stained or unstained, appear solid; but this may be because of the impossibility of seeing them in the greatest detail. In sections of flagellates from both the Madagascar and Mauritius termites they appear as vesicles, whether stained or not (fig. E, 4). The rim is narrow and sharply defined, the interior clear. The most typical form, when stained, has a deep-stained bar at the periphery, often extending halfway around the circumference. A granule appears in some and can be recognized as an optical section of the bar. Many of the spherules appear simply vesicular, showing no granule or bar. They range in diameter up to about  $1.5\mu$ , sometimes more; and in sections all the bodies, of such size, observed in the prenuclear endoplasm were spherules. The striking

morphological similarity between these vesicles and certain Golgi bodies of the dictyosome type must occur to the reader. Though their chemical reactions are not like those of the classic Golgi apparatus, they could be homologized with that structure as logically as can the parabasal apparatus, which resists acetic acid equally well but which Duboseq and Grassé (1933, etc.) have maintained is the Golgi apparatus in certain flagellates.

In larger specimens from *Neotermes howa* of Mauritius the prenuclear endoplasmic bodies are diminished in size and in relative abundance. What there are for the most part are densely crowded behind the rostrum and along the periphery of the endoplasm.

In the posterior part of the body the endoplasm is altogether different in appearance. It contains an abundance of wood fragments; spherules, many of which have a diameter of 2–3 $\mu$ ; and small bodies that have the shape of rods; isolated granules, or pairs and chains of granules. The occurrence of a great abundance of spherules (fig. E, 1), which do not stain but appear brownish in some preparations, is characteristic but not universal. They evidently correspond to the neutral red staining spherules of *Trichonympha sphaerica*.

#### THE PARABASAL APPARATUS OF TRICHONYMPHA

An account of the parabasal bodies of *Trichonympha* was given by me in 1932. Since then, Cleveland *et al.* (1934) have described the parabasal apparatus in a number of species of *Trichonympha* in *Cryptocercus punctulatus*. Improved techniques, particularly the reduced silver method of Bodian (Cole and Day, 1940), have revealed more detail in the structure.

The parabasal bodies, which are cordlike in most species of *Trichonympha*, are continued anteriorly by parabasal filaments to the region at the base of the rostrum. Before terminating the filaments usually run directly beneath the layer of basal bodies of the flagella for a distance that varies in different species. Their anterior connection is a matter of some uncertainty. They can be traced clearly to the region of the circular fissure, where they are applied to the posterior end of the rostral tube, but I cannot be certain that they may not extend into the rostral tube.

Duboseq and Grassé (1927b) were the first to describe the filaments attaching the parabasal cords to the rostrum. They observed them only in certain favorable preparations of *T. chattoni* fixed in Champy's fluid. In none of their figures, however, are the filaments shown clearly or differentiated unmistakably from the flagellar plates. I have seen the parabasal filaments especially clearly in certain protein silver preparations that had been fixed in Hollande's fluid, formolacetic, Bouin's fluid, and a mixture similar to Hollande's except for the use of zinc acetate instead of copper acetate. Those in the three species of *Trichonympha* from *Zoötermopsis nevadensis* (fig. F, 1–4) were revealed on a slide prepared with the last fixative. But in all series it is only occasional specimens that show the structures well.

In *Trichonympha grandis* the parabasal filaments are in the outermost endoplasm, close to the layer of basal granules, for a distance often as far posterior as the anterior end of the pyriform nucleus (fig. G, 2). This arrangement is indicated by Cleveland *et al.* (1934) in figure 198, plate 32. It is typical of the species, rather than the situation shown in their diagram, text figure 28, in which the parabasal filaments are for all their length, until they enter the rostral tube, at an appreciable distance away from the layer of basal granules. Frequently the parabasal filaments leave the peripheral endoplasm all at one transverse level, giving a symmetrical, conical figure (fig. G, 2). The boundary of this figure appears in optical section of

protein silver preparations as a heavy line, which continues anteriorly into the rostrum. Here its substance constitutes the rostral tube. But in surface view of the conical figure I have been able to follow the filaments themselves only as far as the circular fissure (fig. G, 2), and have not been able to detect the parabasal lamella that Cleveland *et al.* found applied to the inner surface of the rostral tube for its full length.

As the filaments pass posteriorly to meet the parabasal bodies they usually do not run longitudinally, but are turned in a spiral that is counterclockwise as viewed from the anterior end (fig. G, 2). The turning is the same in direction as in the laeotropic spiral of the parabasal body of devescoviniid flagellates.

The parabasal bodies stain deeply in protein silver preparations, and the place where they are demarcated from the filaments is distinct. In my preparations of *Trichonympha grandis* from *Cryptocercus punctulatus* of northern California, that place always corresponds to what is shown by Cleveland *et al.* in figure 178, plate 30, representing *T. acuta*. The anterior ends of the parabasal bodies are posterior to the middle of the nucleus, and sometimes the entire length of the cords is posterior to the nucleus. In the specimens I have studied the cords proper do not extend anterior to the nucleus as indicated by Cleveland *et al.* in *T. grandis*. The individual parabasal bodies are usually rather closely spiralled (fig. G, 2), as shown by Cleveland *et al.* in both *T. grandis* and *T. acuta*.

In *Trichonympha campanula*, *T. collaris*, and *T. sphaerica* (fig. F, 1-4) the parabasal bodies are similarly extended to the base of the rostrum by filaments which are close to the layer of basal bodies. In *T. campanula* (fig. F, 1, 2) both the filaments and the parabasal cords are more or less closely spiralled. Some parabasal cords that are interiorly situated are applied to the nuclear membrane, but the filaments of these are, like the others, in the peripheral endoplasm. In *T. collaris* (fig. F, 4) often all of the grouped parabasal cords are in contact with the nucleus; sometimes part of them are free of contact.

Interior to the conical figure formed by the anterior parts of the parabasal filaments of *Trichonympha grandis* is a group of other filaments (fig. G, 1). These form a central columnar figure of variable breadth. The filaments meet the anterior part of the nucleus, which is narrowed and drawn out, then pass anteriorly over the surface of the nucleus, all round its circumference. Beyond the posterior end of the nucleus, the filaments continue free in the cytoplasm. They are slender and more or less straight, in contrast to the stout, spiralled parabasal cords which surround them. The place that the filaments reach posteriorly is usually somewhat short of the posterior ends of the parabasal cords.

The filaments described in the preceding paragraph form a group which corresponds to the nuclear sleeve described by Cleveland *et al.* (1934). I have been unable to ascertain that there is a membrane uniting them or associated with them. Their continuation posterior to the nucleus was not previously reported. I have seen it in a considerable number of iron-haematoxylin stained preparations fixed in Heidenhain's Susa fluid or Zirkle's copper dichromate mixture, in which the axostylar filaments of other hypermastigotes show well.

Filaments comparable to those described above in *T. grandis* are grouped interior to the parabasal apparatus of *Trichonympha alga* (Cleveland *et al.*, 1934, pl. 30, fig. 180). In the latter species the nucleus is posterior to the whole length of the parabasal cords. Enclosed by the parabasals is a columnar structure, consisting of rather stout filaments. After reaching the nucleus these lie against the membrane, and round it posteriorly; they do not extend free beyond the nucleus as do those in *T. grandis*. In Mallory stained specimens the filaments that come in contact with

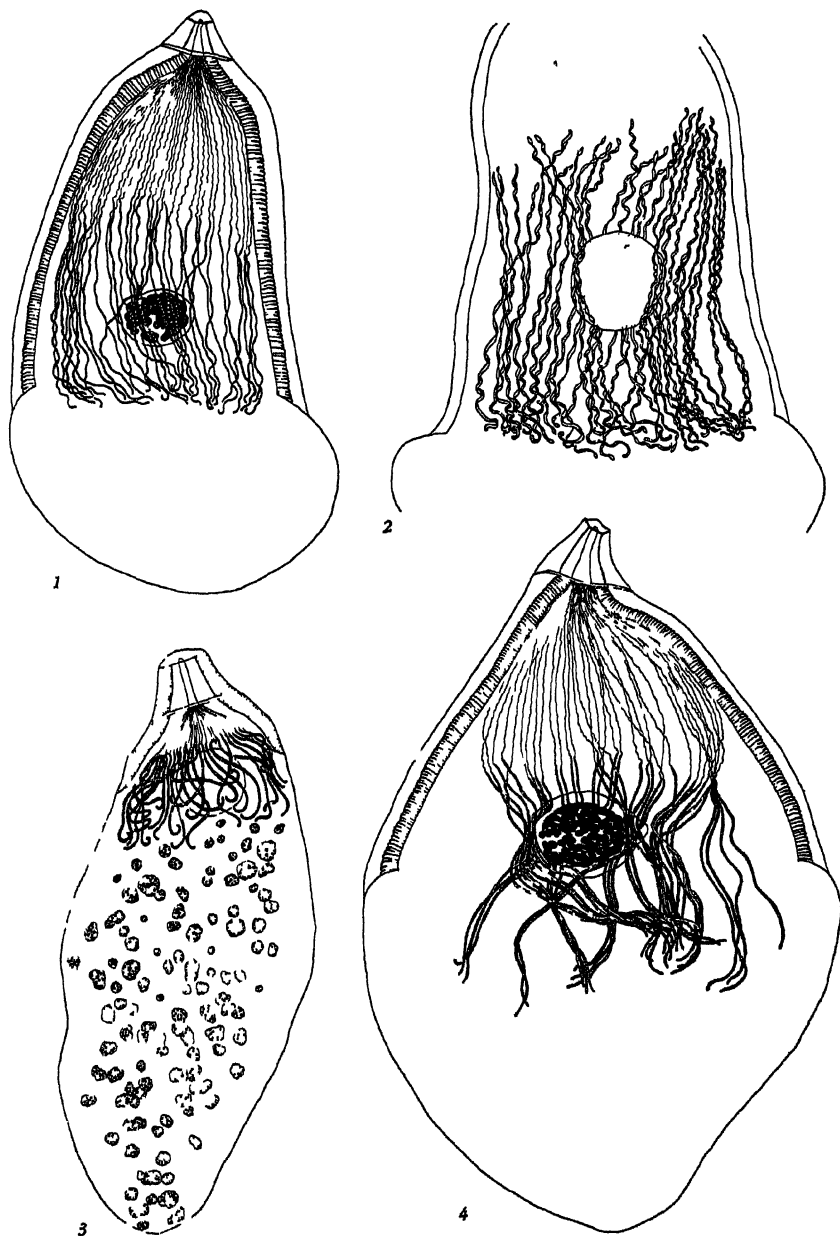


Fig. F. Parabasal apparatus in the species of *Trichonympha* of *Zootermopsis nevadensis*. 1. *T. campanula*. Parabasal cords moderately spiraled, parabasal filaments all situated peripherally in endoplasm and closely spiraled. 2. *T. campanula*. Parabasal cords closely spiraled, two edges blackened by silver. They are shown as seen in the lower half of the body, those over the nucleus being omitted except for some at the edges. Cords in a central group adhere along the nuclear membrane, others are free of the nucleus. This is the typical arrangement in the species. 3. *T. sphaerica*. Parabasal cords moderately sinuous, with filaments to base of rostrum. 4. *T. collaris*. Most of the cords come in contact with the nuclear membrane, and leave the filaments deeper in the endoplasm than in *T. campanula*. Posterior to the nucleus the parabasal cords are in groups. 1, 4, fixation lead acetate substituted for copper acetate in Hollande's fluid; stain protein silver.  $\times 450$ . 2, 3, fixation Gelsei, stain protein silver.  $\times 680$ .

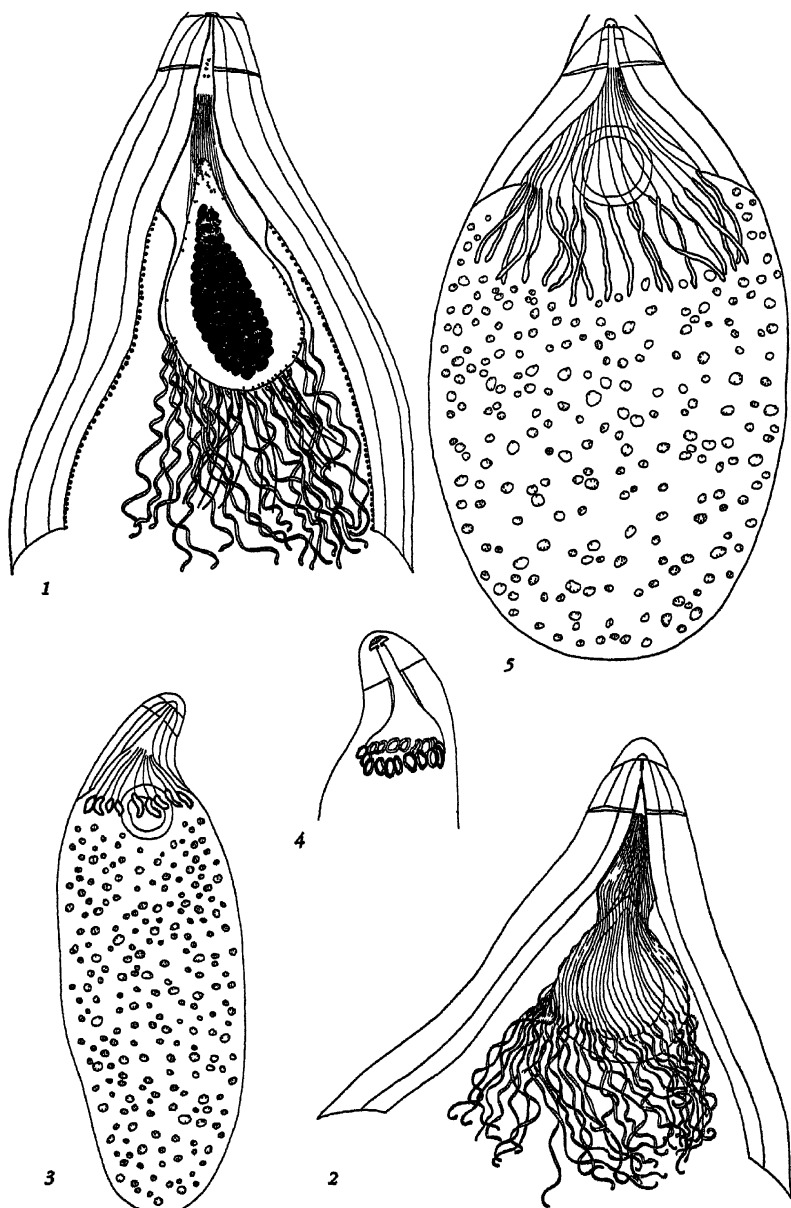


Fig. 6. Parabasal apparatus and certain other structures in species of *Trichonympha* of *Cryptocercus punctulatus*. 1, 2. *T. grandis*. 1 is an optical section through the nucleus, and the parabasal structures that pass over the nucleus are omitted. Meeting the anterior part of the nucleus are fibrils that originate anteriorly in the peripheral endoplasm posterior to the circular fissure. These fibrils continue posteriorly, are applied to the nuclear membrane, and extend free posterior to the nucleus. 2 is a representation of the parabasal cords and parabasal fibrils. The fibrils are in contact with the nuclear membrane, and anterior to the nucleus enter the peripheral endoplasm, continuing in a conical figure to the region of the circular fissure. Within the group are several fibrils that meet the anterior end of the nucleus. 3, 4. *T. parva*. Parabasal bodies appearing as more or less elongated rings. Part of the parabasal filaments extending anteriorly are shown in 3. Endoplasmic spherules shown in 3. 5. *T. chula*. Parabasal bodies elongated, connected by filaments to rostral tube in region of circular fissure, endoplasmic spherules. 1. B. PS.  $\times 800$ . 2. Su. H.  $\times 800$ . 3. Holl. PS.  $\times 1275$ . 4. Formolacetic PS.  $\times 1700$ . 5. Formolacetic PS.  $\times 1275$ .

the nucleus are clearly differentiated from the parabasal apparatus. The parabasal bodies (but not the parabasal filaments) are orange, whereas the filaments, which are not very sharply defined, are blue. Together with the blue layer that appears around the nucleus, the appearance is much like that of a membranous sleeve with ridges. But staining in iron-haematoxylin, in which the structures are sharply defined, does not suggest the correctness of that interpretation.

In *Trichonympha quasilla* (fig. D, 2), the filaments that extend between the region of the base of the rostral tube and the anterior end of the nucleus are comparable to the nuclear suspensory filaments of *T. alga* and *T. grandis*. They have not been traced beyond the anterior end of the nucleus in *T. quasilla*, in which the parabasal cords form a corbule.

In many specimens of *T. parva* the parabasal bodies are circular or elliptical in outline (fig. G, 4). The rim impregnates deeply in preparations made with protein silver, and the interior is more or less clear. The parabasals are arranged in a circle around the nucleus and are continued by filaments to the region of the rostral tube. In other specimens of *T. parva* the parabasals are more elongated (fig. G, 3), and this shape grades into that of the much elongated parabasals of *T. chula* (fig. G, 5) through forms belonging to one or the other species. In both species the parabasals are rimmed in protein silver preparations by an impregnated, heavy line, appearing as a ring more and more drawn out (fig. G, 3-5). The long parabasal bodies of *Trichonympha grandis* similarly are bordered by two heavy black lines, which twist and cross in correspondence with the shape of the parabasals of this species (fig. G, 2). I have observed a similar structure in silver stains of the parabasals of *T. chattoni*, *T. turkestanica*, *T. campanula*, *T. collaris*, and *T. sphaerica*; and evidently it is universal in *Trichonympha*. In some specimens the parabasals appear solid black, or irregularly granular or vacuolated, but existence of the structure described above can often be recognized even in them. The difference in appearance of the parabasal bodies in protein silver and in Schaudinn-iron-haematoxylin preparations is striking. In the latter, as was described in the account of *T. turkestanica* (p. 192) there is a well-defined filament along one edge of each cord, but there is no indication of the rim that impregnates with silver.

In this parabasal structure there is a similarity to what has been described by Duboscq and Grassé (1925a, 1928, 1933) in *Holomastigotes elongatum* and *Spirotrichonympha flagellata*. They were the first to use a silver method (Da Fano) in study of the parabasal apparatus of hypermastigotes. In the former species they described a cap of deep-staining substance bordering a vesicle of non-staining material. I have seen the same picture in the parabasal bodies of *Holomastigotes elongatum* from *Reticulitermes hesperus*, prepared by the protein silver method. Parabasals of similar structure were found by Duboscq and Grassé (1933, pl. 9, fig. 3) in silver impregnated *Spirotrichonympha flagellata*. In that hypermastigote the deeply impregnating substance forms a partial or often a complete ring around the other substance. The flagellates of the *Microjoenia*-type from *Reticulitermes lucifugus* (*M. hexamitoides* Grassi), which were considered by Duboscq and Grassé (1928) to be young stages of *Spirotrichonympha*, the parabasals in osmic and silver preparations show a similar structure. They are arranged in a circle around the nucleus and are extended by parabasal filaments toward the anterior end of the body. Brown (1930) described similar connections in *Microjoenia* forms from *Reticulitermes hesperus* named by him *Torquenympha octopus*. In both structure and arrangement of the parabasal apparatus of this species of *Microjoenia* there is a similarity to that of *Trichonympha parva*.

The usually numerous, rounded parabasal bodies that border the spiral flagellar



bands of Holomastigotidae (Spirotrichonymphidae) seem to constitute a very different sort of apparatus from that in Trichonymphidae. But consideration of the situation in *Microjoenia* and *Trichonympha parva*, seems to point to a closer relationship between the parabasal structures in the hypermastigotes of those two families than might at first be suspected. In *Microjoenia*, the holomastigotid parabasal apparatus is represented at its simplest, and increasing complexity may be traced in the other genera of the family; in *Trichonympha parva*, there is the simplest form of the trichonymphid apparatus.

The significance of the parabasal structures in the comparative morphology of *Trichonympha* has been commented upon by Cleveland *et al.* (1934) and by me (1932). The configuration of the apparatus is rather constant within a species, and there are marked differences among species or groups of species. It is one of the most valuable characteristics for systematic purposes.

Of the species of *Trichonympha* in *Cryptocercus punctulatus*, *T. parva* and *T. chula* (fig. G, 3-5) have the most generalized structure of the parabasal apparatus. In them the stout, rounded, or moderately elongated parabasal bodies are in the peripheral region of the endoplasm in a circle around or posterior to the nucleus (fig. G, 3-5), and normally are free of contact with the nuclear membrane. In *T. grandis* the parabasal bodies are more numerous and more elongated than in the other species (fig. G, 1, 2). Anteriorly, the parabasal filaments are situated in the outermost endoplasm; posteriorly they turn inward and continue more or less in contact with the nuclear membrane. The parabasal cords in their anterior part are also more or less in contact with the nucleus. The parabasal apparatus is similarly arranged in *T. acuta*, and in the flagellates designated as *T. okolona* and *T. lata* (Cleveland *et al.*, 1934, pl. 30). The trichonymphas in *Cryptocercus punctulatus* that have the wide inner cap and the very distinct, long central rod within the rostral tube are of two types as regards the parabasal arrangement. In one, *T. algoa* (Cleveland *et al.*, 1934, pl. 30, fig. 180), the entire length of the parabasals is anterior to the nucleus; these do not even at their ends come in contact with the nuclear membrane. (In the last point the parabasal apparatus of *T. lighti* (fig. B, 2) is unlike that of *T. algoa*.) Other flagellates with a rostrum of the *T. algoa* type are present in *Cryptocercus punctulatus* from northern California. In them the parabasal cords are isolated from one another and usually are situated entirely in the most peripheral endoplasm around the region occupied by the nucleus.

Of the parabasal apparatus in the species of *Trichonympha* of termites, the most generalized structure is that in *T. sphaerica* (fig. F, 3) and in the species of the *T. chattoni* group—*T. chattoni*, *T. divexa*, *T. zeylanica*, and *T. tabogae* (fig. A, 1-7, 9, 10). In these the arrangement of the parabasal bodies is comparable to that in *T. chula* and *T. parva*, except that often some of them lie adjacent to the nuclear membrane, and they are longer. *T. subquasilla* (fig. A, 8) differs in the gathering of the parabasal filaments in the central endoplasm, so that the cords are in a group at their origin instead of beginning near the layer of basal granules of the flagella. At most only part of them come in contact with the nuclear membrane. In *T. lighti* (fig. B, 2) the anterior grouped arrangement of the parabasal bodies is similar to that of *T. subquasilla*, but most of them are applied at their extreme ends to the posteriorly shifted nucleus.

In all the species mentioned above the parabasal bodies are essentially separate from one another; but there is manifested in several species a tendency toward parallel application of greatly elongated parabasals. The parabasal apparatus is a more or less cylindrical structure open at both ends in *T. saepicula* (fig. B, 1) and *T. ampla* (fig. C, 3). The nucleus is enclosed within the group, but is usually not at

the extreme posterior end. In *T. quasilla*, *T. corbula*, and *T. teres* (fig. D) a corbule is formed by application of the terminal parts of all the cords to the posterior nuclear membrane. It is seldom in these species that any cords are either free of entry into composition of the corbule, or extend into the endoplasm posterior to the nucleus. In all the species heretofore discussed, including those in *Cryptocercus punctulatus*, the parabasal bodies begin at approximately one transverse level. This level is most often, though not always, in the neighborhood of the posterior terminus of the flagellar plates. A unique arrangement is that of *T. peplophora* (fig. E, 1), in which the parabasal bodies begin near the layer of basal granules at various positions along the longitudinal axis. The parabasal filaments, which extend in the peripheral endoplasm to the base of the rostrum, are therefore of various lengths. All of the species of *Trichonympha* of termites mentioned in this paragraph have characteristic endoplasmic spherules, in common with *T. chula* and *T. parva*.

The *Trichonymphas* without these spherules are all those in *Cryptocercus punctulatus* except *T. chula* and *T. parva*, and five or six species in termites. Except *T. sphaerica*, all those known in termites other than Kalotermitidae belong in that group. They have in common too, along with *T. grandis* and related species in *Cryptocercus punctulatus*, a relatively long flagellated zone. The parabasal apparatus has a variety of forms. The most generalized condition is that in *T. grandis* and *T. campanula* (fig. F, 1, 2), in which the cords are isolated from one another. Contact with the nuclear membrane is established by some of those in *T. campanula*, and in *T. grandis* most of them come more or less into such contact. Arrangement of the cords in small groups, usually with contact with the nuclear membrane, characterizes *T. collaris* (fig. F, 4). A parallel, cylindrical grouping of the cords, forming an open tube within which the nucleus is enclosed, occurs in *T. magna* of *Porotermes adamsoni* (Kirby, 1932, pl. 28, fig. 43). In *T. agilis*, there is a corbule, as in *T. quasilla*. Finally, *T. turkestanica* (pl. 21, fig. 112) resembles *T. peplophora* in the origin of the parabasal bodies near the layer of basal granules at various levels in the flagellated zone.

The foregoing account does not pretend to establish any sort of taxonomic grouping of species of *Trichonympha*. Before that can be undertaken profitably, we need information about more species.

The parabasal bodies of *Trichonympha campanula* are sometimes branched (fig. F, 2). I have observed this in certain ones of the set in many specimens. One or two rami are given off generally in the middle or the posterior part of the cord. The rami usually extend posteriorly as far as the other parabasal structures. Consequently there may be more ends at the terminus of the apparatus posterior to the nucleus than there are parabasal filaments extending to the base of the rostral tube.

#### SOME OBSERVATIONS ON THE ACHROMATIC FIGURE IN THE DIVISION OF TRICHONYMPHA

I have not attempted to make a study of the complete division process in any species of *Trichonympha*, nor to work out at all the course of events in the chromatic figure. I have, however, given careful attention to certain features of the division process, initially in order that I might be better able to interpret certain structures in the non-dividing flagellates.

I was interested first in ascertaining whether the two crescentic bodies at the anterior end of the rostral tube, and the central rod in the rostral tube of *Trichonympha turkestanica*, could correctly be named centrioles. This led to an investigation of the relationship of the poles of the division figure, in this and

other species, to other structures. Finally, some observations were made on the origin of the new rostral flagella and the fate of the parabasal apparatus.

Apparently, in species of *Trichonympha* the position of the poles of the division figure is not uniform. Previously published reports indicated as much, but it was supposed that some of them were incorrect. I have observed the division process in *T. turkestanica*, *T. collaris*, *T. campanula*, *T. sphaerica*, *T. chattoni*, *T. lighti*, *T. ampla*, and *T. peplophora*. These species show two different situations and that in *Trichonympha* of *Cryptocercus punctulatus*, as described by Cleveland *et al.* (1934) is unlike either of them.

The first problem to arise was the behavior of the central rod in the anterior part of the rostral tube of *Trichonympha turkestanica*. Cleveland *et al.* (1934) identified as part of a centriole the rod in a similar position in *Trichonympha grandis* and other species of *Cryptocercus punctulatus*, and justified this identification by the role the rod plays in the development of the spindle. According to their description, the rod meets at the anterior end of the rostral tube that one of the two symmetrically placed crescentic bodies which is the proximal end of this elongate centriole. The other crescentic body represents the second centriole; in the interphase, only this proximal end of it is present. They described it as developing a rod at the beginning of division, before separation of the halves of the rostral tube. The rod was reported to elongate laterally, more or less perpendicularly to the other centriole, thus passing through the substance of the ectoplasm of the rostrum, under the cap and along the anterior margin of the flagellated region. When this new rod attains the length of the other, astral rays grow out from the distal ends of both, and some of them meet and form the primary spindle. This spindle is at first at an angle to the longitudinal axis of the rostrum, with one centriole rod in that axis. As the rostra separate, the new centriole turns to become parallel to the other. Finally the distal ends of the two rods, which are connected by the spindle, come to coincide spatially with the posterior ends of the separated halves of the rostral tube. (Cleveland *et al.*, 1934, text figs. 32, 33.)

The central rod in the rostral tube of *Trichonympha turkestanica* (pl. 21, figs. 104-108) is generally shorter than that in the species in *Cryptocercus punctulatus*; its length is less than half that of the rostral tube, whereas in the others it extends, according to the species, from half the length or less to almost the full length of the tube. The drawings by Cleveland *et al.* indicate some variation within a species in the length of this rod relative to the tube. In all species in *Cryptocercus* in which division is shown, the ends of the spindle, after the final position is assumed, are anchored near the circular fissure, that is, at the posterior end of the rostral tube. In order to maintain contact with the spindle poles, the centrioles that do not extend full length originally must, then, elongate to this full length. The central rod in *Trichonympha turkestanica* has a constant relative length, and this is maintained in the rods that are visible in all the phases of mitosis.

In the process of binary fission, the earliest change in the rostrum of *T. turkestanica* is a divergence of the posterior ends of the halves of the rostral tube, and along with this, separation of the flagellar systems. The anterior ends of the tube halves remain close together until after those halves have turned in opposite or nearly opposite directions. In this separation the rod passes unaltered with one half of the tube (fig. H, 1), and not until these structures have migrated entirely apart does a new one differentiate in the other half (fig. H, 2). The new rod is at first more slender and paler than the other. It may at first have a direct connection with the crescentic body, but later, as in the old one, no direct connection is apparent.

At no time is there any relationship between these central rods in *T. turkestanica*, and the astral rays, or central spindle. I have not found the earliest phases in development of the achromatic fibers; but, when they have been seen, and throughout the mitotic process, the astral rays and spindle fibers are associated directly with the posterior ends of the rostral tube halves (fig. H, 2, 4). The connection, however, is in some instances not direct. Spindle fibers and astral rays stop short of actual union with the rostral tube (fig. H, 5, 6c).

In the position of the poles of the central spindle, *T. turkestanica* is in agreement with *T. agilis*, *T. collaris* (fig. I, 3), *T. campanula*, and *T. sphaerica* (Kofoid and Swezy, 1919). I agree with Kofoid and Swezy about the position of the spindle in the last two species (fig. I, 1, 2).

After the halves of the rostral tube of *T. turkestanica* have separated, in the prophase before the extranuclear spindle has attained its full length or reached its final position in relation to the nucleus, the new halves of the rostral tube begin to grow out (fig. H, 2, 4). They are lamellalike structures, and, after extending about half the length of the old rostral tube half, are invariably curved sharply away from it. From the earliest appearance of this lamella short new flagella take origin in a dense group along its anterior part. The size reached by the lamella and new flagella in the prophase is not greatly exceeded in the later phases of mitosis; further development takes place after plasmotomy (fig. H, 6b).

In the three species of *Trichonympha* in *Zoötermopsis nevadensis*, I have also observed the new outgrowing rostral tube halves, which, as in *T. turkestanica*, are not related to the poles of the spindle (fig. I, 1-3). In the prophase and anaphase figures studied, the new rostral tube half, as in the other species, is a narrow lamella, curved away from the old tube half, and bearing new flagella on the anterior part of its concave side.

Dubosq and Grassé (1927a, b) observed in mitosis a limited number of *Trichonympha chattoni* from *Glyptotermes iridipennis*. In regard to the achromatic figure, they drew the significant conclusion that in this species "the rostrum is not a centrobipharoplast and, after duplication, it does not occupy the poles of the spindle" (1927a). The poles of the spindle, at which were observed "attraction spheres" and asters, are shown in two figures to be entirely separate from the rostrum and the flagellar rows; in another figure they seem to be in contact, or nearly so, with the anterior ends of the rostra.

I have seen a large number of division figures of *Trichonympha chattoni* from the same host species, and have been able to find the explanation of these figures by Dubosq and Grassé, which seemed at the time of publication to be so at variance with the situation in other species of *Trichonympha*. In *T. chattoni* I have seen no central rod in the rostral tube.

The earliest extranuclear change that I have noted in *T. chattoni* is the separation of the halves of the rostral tube (fig. J, 1, 2). In early stages of this separation, the anterior ends of both halves remain, as originally, covered by the same cap; the posterior ends of the tube halves come to lie farther apart than the anterior ends. Eventually the tube halves extend in almost opposite directions, with the anterior ends still rather close together, and connected by a strand (fig. J, 5). Meanwhile, from the anterior ends of each of the tube halves, a structure grows out, extending at first posteriad between the two separating but more or less parallel halves (fig. J, 3, 4). This structure is at first filamentous, then a rod, then broadens to a narrow lamella. Before long, short new flagella begin to develop from one surface of it (fig. J, 10, 12). The lamellae ultimately acquire a bend near or distal to the middle, and the new flagella arise from them mostly anterior to the bend.

It may be contended that these outgrowths in *T. chattoni* are homologous with the centrioles in the roach *Trichonymphas*, and are represented in the interphase only by the crescentic bodies at the anterior ends of the rostral tube halves, neither one being elongated. There is certainty, however, in the homology with the new

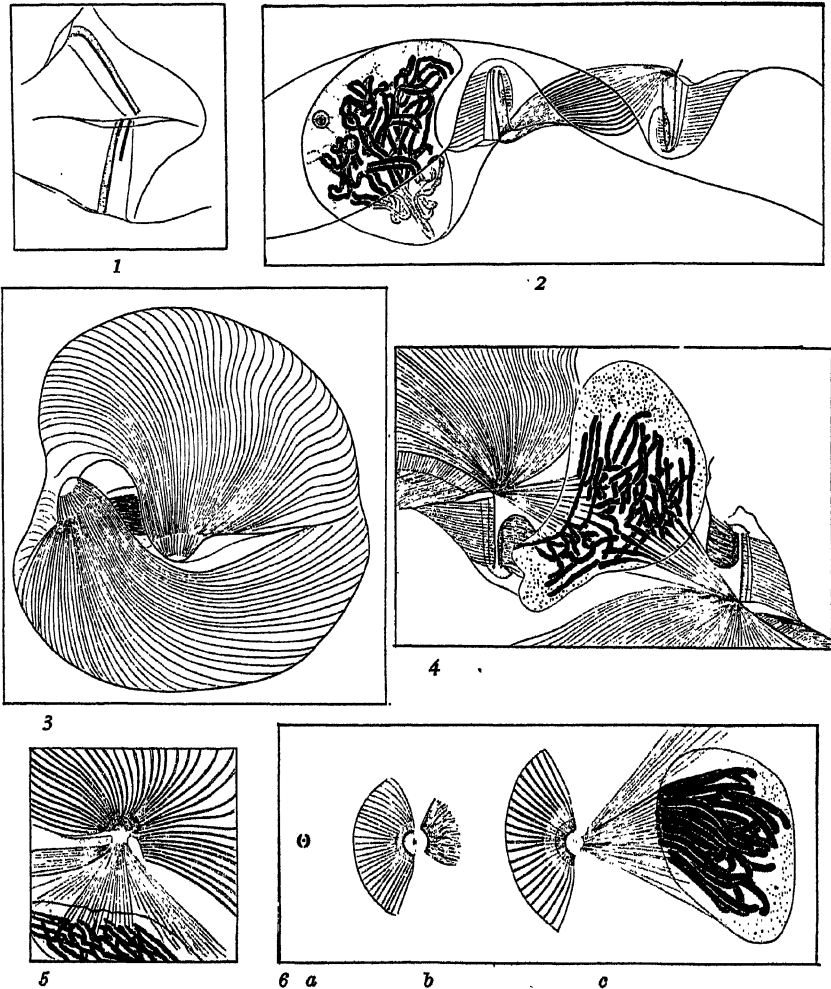


Fig. H. Division of *Trichonympha turkestanica* from *Anacanthotermes ochraceus*. S.H. 1. Separating halves of the rostral tube; these are still close together anteriorly, but diverge widely posteriorly. The central rod in the anterior part of the rostral tube has passed intact with one half, and no central rod is visible in association with the other half. The nucleus of this specimen (not shown) is in the very early prophase, with loosened spireme threads.  $\times 1325$ . 2. The halves of the old rostral tube are separated and turned in opposite directions; in each is a central rod, the retained old one in one half, a new one in the other. The stout, fibrillar spindle is well developed; its poles connect to the posterior rods of the old rostral tube halves. The nucleus is in early prophase, with chromosomes split and paired.  $\times 880$ . 3. Surface view of flagellate in a division stage similar to that of figure 2. There are two rostral tube halves and the flagellar plates are distributed into two groups.  $\times 340$ . 4. Nucleus in early anaphase. The chromosomal fibers are shown; with the spindle fibers they connect to the posterior ends of the separated old rostral tube halves. The central rods, shown in the anterior parts of these halves, have no relation to the spindle fibers. A new developing rostral tube half, sharply curved and bearing on one side new flagella, is shown adjacent to each old rostral tube half. From the poles of the spindle and the posterior ends of the rostral tube halves radiate fibrils that lie deeper in the cytoplasm than the plates, and are

halves of the rostral tube as observed in *Trichonympha turkestanica*. In the latter species, there is also present a central rod, which corresponds in position, but not in role in mitosis, to the rod designated by Cleveland as an elongated centriole in roach *Trichonymphas*; and from the standpoint of the position of the division figure there can be no question of regarding the new rostral tube halves of *T. turkestanica* as centrioles.

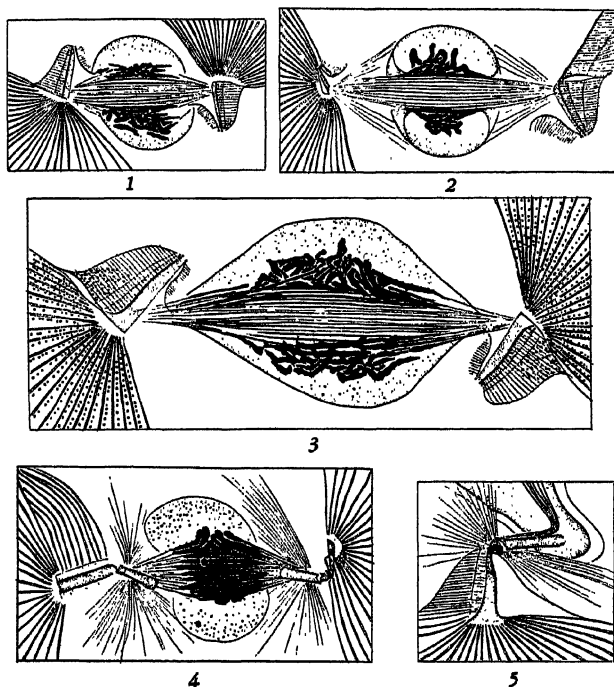


Fig. 1. Division process of *Trichonympha*.  $\times 880$ . 1. *T. sphaerica* from *Zoötermopsis angusticollis*. The poles of the spindle come into relation to the posterior parts of the old rostral tube halves. New rostral tube halves, with short outgrowing flagella, are present. Su.H. 2. *T. campanula* from *Z. angusticollis*. Spindle lying in groove in nucleus, poles meeting posterior ends of old rostral tube halves. New rostral tube halves bent and bearing short new flagella. Su.H. 3. *T. collaris* from *Z. angusticollis*. Relation of poles of spindle to old rostral tube halves the same as in the preceding two species. Granules between the plates characteristic of this species. Su.H. 4, 5. *T. pepiophora* from *Neotermes howa*, Mauritius. S. H. 4, Anaphase. The new rostral tube halves are distinctly band-formed, extended toward the spindle, and the fibers of the asters and spindle center in a point near the anterior part of these. 5. Anaphase. Only half of division figure shown. The new rostral tube half passes horizontally, and is paralleled by fibers of the spindle. The astral rays and spindle fibers end near the anterior end of the new rostral tube half, as in *T. chattoni*, not at all as in *T. collaris*, *T. sphaerica* and *T. campanula*.

*Description of Fig. H continued.*

evidently the parabasal fibrils.  $\times 880$ . 5. The nucleus is in the metaphase; only half of the division figure is shown. The rostral tube half, in semicircular form, is viewed vertically, and the focus is on the most posterior part. The plates radiating from its vicinity are the body plates. Radiating from the region of the posterior end of the old rostral tube half are shown chromosomal fibers and astral rays.  $\times 880$ . 6. After division of the body has been completed. The rostral tube structure is viewed vertically. 6a is focused on the crescentic bodies at the anterior end of the rostral tube halves, both old and new; between them (although it is at a slightly deeper focus) is shown the anterior end of the central rod. 6b is focused at the level of the posterior end of the central rod. The old rostral half shows the crescentic tube, the central rod, the ectoplasmic layers, and the radiating plates. The free parts of the flagella are omitted. At the right is the new rostral tube half, from the outer side of which originate short new flagella. 6c is focused at approximately the posterior end of the old rostral tube half, and shows the relation to this of the chromosomal fibers and astral rays. The nucleus is in the telophase, and the chromosomes are all directed to one pole.  $\times 1325$ .

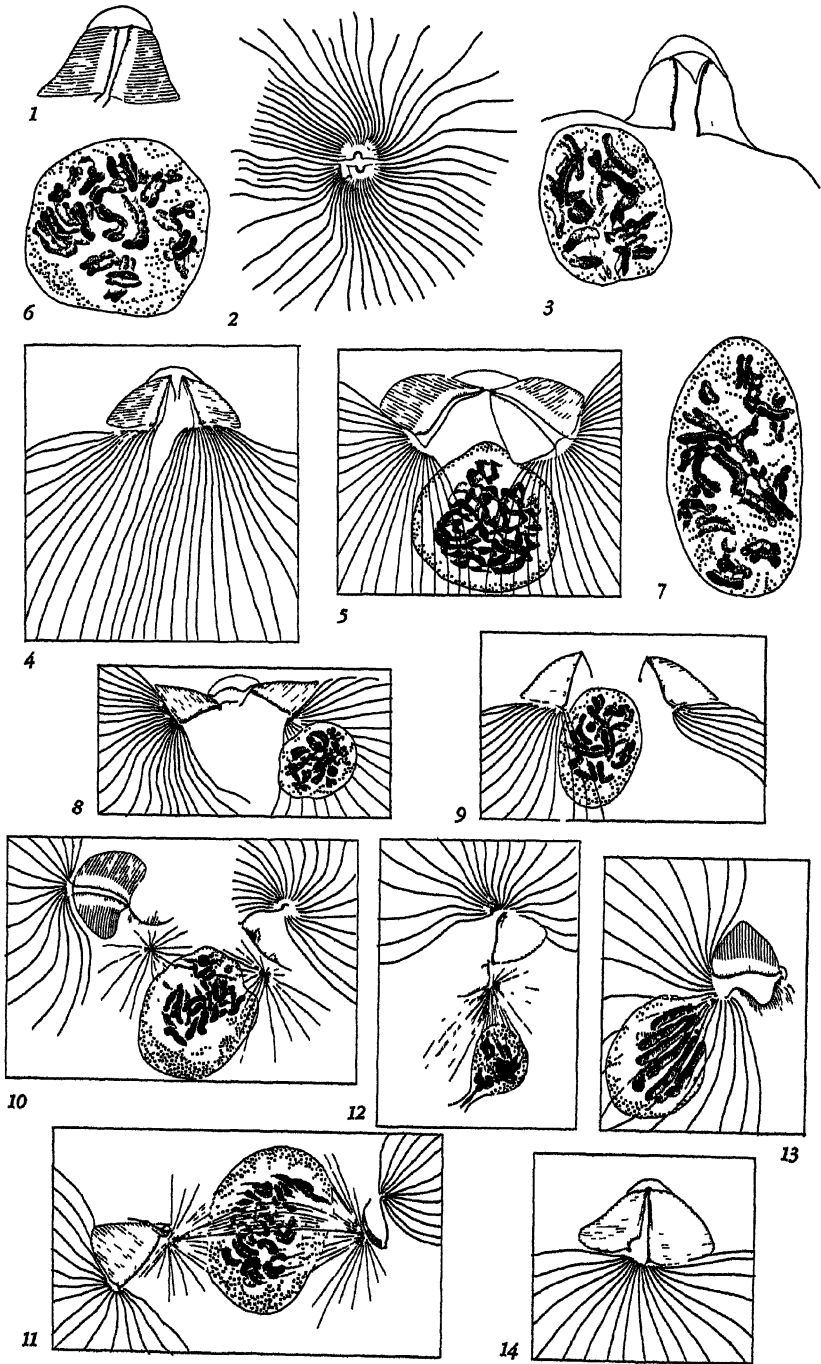


Fig. J. Division process of *Trichonympha chattoni* from *Glyptotermes iridipennis*. S.H. 1. Beginning of separation of the halves of the rostral tube. 2. Vertical view early in the separation of the halves of the rostral tube. The focus is on the posterior ends of the semicircular rostral tube halves, and the plates shown are those of the body, not the rostrum. 3. Rostral tube halves separated along whole length, anterior ends of both surmounted by a common cap. The filaments extending

Nevertheless, the new rostral tube halves in *Trichonympha chattoni* are related to the division figure. In this there is a striking difference from the instances described of division in *T. turkestanica*, *T. collaris*, *T. campanula*, *T. sphaerica*, and *T. agilis*, in which the poles of the spindle are adjacent to the posterior ends of the parent rostral tube halves.

In the earlier stages of separation of the old rostral tube halves in *Trichonympha chattoni*, the new rostral tube halves are still small and their posterior ends are close together (fig. J, 4, 8). A region of somewhat denser-appearing cytoplasm has been seen in such stages between the posterior parts of the latter. There is no indication of fibrils in this denser area, but it may represent the region of early development of the astral rays and spindle. In later prophase stages, when the new rostral tube halves are farther apart, and the chromosomes are still in pairs, astral rays appear. The centers of radiation are far from the ends of the old rostral tube halves, and are associated with the new rostral tube halves; they are situated not at the ends but near the region of the bend (fig. J, 10, 11, 12). In at least some instances it is clear that the centers of radiation are not in the substance of the new rostral tube halves themselves; but nevertheless a constant position in relation to the latter is usually maintained. Astral rays have been seen in earlier stages than have spindle fibers. In later prophases and subsequent stages there is a fibrillar spindle (fig. J, 11); and generally the new rostral tube halves are extended in the direction of the spindle, as if fixed at the anterior ends, but drawn toward one another by an elastic connection near the posterior ends. The old rostral tube halves may extend in almost opposite directions (fig. J, 12). In addition to the astral rays that extend freely into the cytoplasm and the fibers of the spindle that extend from pole to pole, there are fibers from the center of radiation that impinge upon the nuclear membrane (fig. J, 11). These correspond to the extranuclear chromosomal fibers described in detail by Cleveland *et al.* in *Barbulanympha*, *Trichonympha*, and other hypermastigotes.

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halves. Nucleus with split, paired chromosomes. 4. Rostral tube halves separating, closer at the anterior than the posterior ends. Between them, growing obliquely posteriorly from the body at the anterior end of each rostral tube half, are filaments which will become the new rostral tube halves. 5. Rostral tube halves separated widely at their posterior ends, close together at their anterior ends. The rostral half on the right side is seen in flat view, the one on the left side in edge view. 6. Nucleus in early prophase, chromosomes have split and chromatids are paired. There are probably 20 split chromosomes. 7. Similar nucleus in early prophase, apparently 20 split chromosomes, 40 in all. 8. Separation of the halves of the old rostral tube, with new rostral tube halves growing out as slender rods, one from the granule at the anterior end of each old rostral tube half. The posterior ends of these rods are close together, and in the region of dense cytoplasm around these ends may perhaps be found the beginning of the spindle. 9. Old rostral tube halves are further separated, and the outgrowing new rostral tube halves are distinct. There may be some development of astral rays and spindle, but the stain was too pale to reveal these fibrils. 10. The old rostral tube half on the left side is seen flat, that on the right side on edge. Close to the granule at the anterior edge of each is a second granule, to which a developing new rostral tube half is connected. Short flagella are developing from the surface of each of these two small bands. Near these new rostral tube halves, but not directly connected to them, are the centers of radiation of astral rays and spindle fibers. 11. The spindle is clear and passes over the top of the nucleus, and the chromosome fibers and astral rays are distinct. The center of radiation at each pole is in close proximity to the middle part of each new rostral tube half, but is not actually in the substance of this band. 12. The new rostral tube half has an angled form, and the granule at its anterior end is separate from, close to, and smaller than the granule at the anterior end of the old rostral tube half. The fibers of the aster and spindle converge at a point very close to the bend of the new rostral tube half. Only half of the division figure is shown. The nuclear division figure is abnormal; the paired chromosome halves indicate a prophase condition, in spite of the anaphaselike construction. 13. After plasmotomy. The new rostral tube half, bearing short flagella, is moving in to parallel the old rostral tube half. The telophase chromosomes are directed toward one pole of the nucleus. 14. After plasmotomy. The chromosomes have disorganized and the flagella and ectoplasmic layers of the new half of the rostrum have reorganized almost completely. The posterior part of the new rostral tube is still, however, not entirely parallel to the old half. Figs. 4, 8, 9, 10, 12,  $\times 860$ ; 1, 2, 3, 5, 11, 13, 14,  $\times 1275$ ; 6, 7,  $\times 1750$ .



In the above-described relationship of the achromatic figure, we find the explanation of the account by Dubosq and Grassé (1927a, b). They failed to observe the new rostral tube halves, but the position they found the spindle to occupy in relation to the old rostral tube halves may well be correct. I have seen figures that are in essential agreement with all of those they made to illustrate this relation.

It is more difficult, however, to understand the figure that they made of the telophase (their pl. 19, fig. 32). In it the two daughter individuals have not yet been separated by cytoplasmic division; but the rostra and nuclei, at opposite ends, appear completely reorganized. If plasmotomy should occur in this specimen, the daughter individuals would not be distinguishable, even immediately afterward, from ordinary interphase specimens. I have seen many specimens of *T. chattoni* following cytoplasmic division, and have been able to recognize them readily by the condition of the nucleus and the rostral tube (fig. J, 13, 14). At first the chromatin of the nucleus is not in its interphase condition, but is in elongated parallel chromosomes directed toward the part of the nuclear membrane upon which fibers from the center of radiation along the new rostral tube half still impinge. The new rostral tube half is turned so as to lie closer to the old one, but still does not lie parallel to it; nor is the new rostral tube half straight. The new flagella are short and the system of ectoplasmic layers and plates has not yet differentiated. Eventually the two tube halves become parallel; but when nuclear reorganization is largely complete, the posterior end of the new tube half may still be separated somewhat from the old one. It must then be supposed that Dubosq and Grassé's telophase represents not a typical condition, but an instance of delayed plasmotomy (if, indeed, there really is a cytoplasmic union).

The division process in *Trichonympha peplophora* (fig. I, 4, 5) is similar to that in *T. chattoni*. In the late prophase, when the two rostral tube halves have separated and moved to opposite sides of the nucleus, new outgrowing rostral tube halves are developed much as in *T. chattoni*. Each is a band of moderate length, roughened in its anterior section by the outgrowth of short new flagella, and extended toward the nucleus in the direction of the axis of the spindle. There is no elbowlike bend as in the new rostral tube halves of *T. chattoni*; the curvature is relatively slight and gradual. The extranuclear spindle is stout and fibrillar, and its poles are centered in asters. The astral rays radiate in all directions, and many fibers come in contact with the nuclear membrane, where they meet chromosomal fibers. The centers of radiation, to the extent that they could be observed in the material available for study, are not marked by granules or other stainable structures. They are located in close proximity to the new rostral tube halves, at a point often about a quarter of the distance from the anterior end. Sometimes there is an observable difference between the part of the new rostral tube half anterior to the point of proximity of the center of radiation of the astral rays and the part posterior to it. A more heavily staining border may be present along the more anterior part, and there may be an apparent interruption of substance between it and the posterior section.

In *Trichonympha peplophora*, as in *T. chattoni*, I have seen no rod in the center of the anterior part of the rostral tube.

The poles of the spindle are related to the new rostral tube halves, rather than to the old ones, also in *T. ampla* and *T. lighti*. I have seen a central rod in the rostral tube in the former species, but not in the latter. In the one division stage of *T. ampla* that I was able to study, a short, deep-stained rod originated near the anterior end of each new rostral tube half and extended separate from it. The spindle fibers met the posterior end of this rod. No such rod, separate from the new rostral tube half, was seen in the more abundant preparations of *T. chattoni* and *T. peplophora*;

the situation in *T. ampla* is probably of basic significance, and should be investigated.

In all the species I have studied the chromosomes form, divide, and migrate to the poles in a manner essentially the same as that described by Cleveland *et al.* in the species of *Trichonympha* of *Cryptocercus punctulatus*. The daughter chromosomes resulting from the split, which occurs early in the prophase, are paired throughout most of the prophase (fig. II, 2; J, 3, 7-9). Each is prolonged at one end by an intranuclear chromosomal fiber. Eventually, beginning at this end, the daughter chromosomes gradually separate, they remain in contact at the other end until the last moment. In stages after fission of the body, they are more or less straight and are applied at one end to the part of the nuclear membrane closest to the rostral tube halves (fig. II, 6c; J, 13).

Preparations of *Trichonympha chattoni* restained in protein silver have been of much value in following the distribution in mitosis of the plates and parabasal bodies. The plates of the rostrum and the body separate into two groups. The two groups are approximately equal; when there is an even number there may be an exact separation; when an odd number, one more plate on one side. Sometimes, however, distribution is unequal. Instances were seen of separation into groups of 21 and 23, and 24 and 22. The parabasal bodies also become distributed into two groups, and they appear to persist intact throughout the process of division. I have seen them clearly at all stages. A similar distribution of the parabasal bodies is shown by Duboseq and Grassé in this species.

New plates and new parabasal bodies do not appear during the entire process of division. In the later telophase stages, the old plates become spread around each of the forming daughter individuals, so that the group of plates is not semicircular as before but may be almost closed in a circle. After plasmotomy, there are then two individuals, each with about half the normal number of plates. New plates and new parabasal bodies, to complete the number, develop after division, but before the nucleus has resumed the typical interphase condition. I have not been able to trace the development of these new structures; but there is evidence that new plates develop interspersed, in groups of one or more, between the old plates.

During the process of mitotic division of *Trichonympha turkestanica* there is deformation, detachment, and fragmentation of the old parabasal cords. This process begins in very early prophase stages; resorption of the fragments is not far advanced even at plasmotomy. The disintegration of the cords is indicated very clearly in protein silver preparations, because every fragment is shown plainly. Throughout mitosis parabasal filaments, extending from the base of the old rostral tube half, may be seen in iron-haematoxylin preparations (fig. H, 4), but they are indistinct in the silver material. Protein silver shows no new parabasal structure, even at the completion of plasmotomy. The material studied at the present time has not revealed whether the parabasal filaments seen during mitosis belong to the old apparatus or are new outgrowths; the former is the more probable situation. Thus there would be a discarding of a part but not all of the old parabasal apparatus, as in *Devescoviniinae*. This situation is unlike what seems to occur in *Trichonympha chula*, *Barbulanympha*, and *Urinympha talea* (Cleveland *et al.*, 1934), and in *Trichonympha chattoni*; in them the old parabasal cords persist intact and are distributed into two groups.

The observations reported here on the achromatic figure in species of *Trichonympha* of termites are neither complete nor conclusive. They do serve, however, to establish certain facts that must be taken into account in shaping our understanding of the division process in flagellates with an extranuclear spindle. (This struc-

ture in polymastigotes and hypermastigotes has often been termed the paradesmose, but is more correctly called the central spindle, as pointed out by Belar, 1926, and Cleveland *et al.*, 1934, or the centrodesmose.) Before general conclusions can be drawn, we need much detailed information concerning the process in different species of polymastigote and hypermastigote flagellates. In the latter group, this information is now being supplied, almost entirely, in the series of admirable papers by L. R. Cleveland. It is of interest in this place to consider how the situation here revealed in species of *Trichonympha* in termites compares with that in related forms.

In all the hypermastigotes in which Cleveland has studied division he has described, at least in that phase of the life history, elongate, sometimes filamentous centrioles. In some genera, elongate centrioles persist without essential alteration of form throughout the interphase. In others, in the interphase, either they are present in altered form or they cannot be recognized.

The structural relationships in *Barbulanympha* are diagrammatic in their clearness, and it is a flagellate of basic importance in this connection. In species of *Barbulanympha*, and also in *Rhynchonympha*, *Urinympa*, and *Idionympha*, two equal-sized rods or filaments are present throughout the interphase. The poles of the spindle are associated with the posterior ends of these two structures (and because of that the latter are considered to be centrioles). *Idionympha perissa* is unique in that the fibers of the spindle are present throughout the interphase between the posterior ends of these elongate centrioles. The presence of these rods, with their clear and convincing relationships to the spindle poles, has stimulated a search for comparable structures in related flagellates.

Cleveland *et al.* (1934) and Cleveland (1935, 1938a, c) were unable to distinguish the centrioles convincingly in the interphase of *Eucononympha*, *Pseudotriconympha*, *Teratonympha*, *Macrospironympha*, *Leptospironympha*, or *Spirotrichonympha*, except that in some of these, paired granules at the anterior ends of the rostral tube or equivalent structures might represent them.

In the first three of these genera, in division, a more or less long strand extends from the posterior end of each half of the rostral tube to the poles of the spindle; and it seemed likely to Cleveland that it continued anteriorly along the rostral tube half to the anterior end. In *Leptospironympha*, a strand connects each spindle pole to a flagellar band, passing to the base of the longitudinal rostral portion and then continuing anteriorly along it. In *Spirotrichonympha polygyra*, in which the four bands are spiraled to the anterior end, the spindle fibers appear to arise directly between two of the bands. Cleveland believed, however, that there is a slender, elongate centriole closely following each of these two bands from the anterior end for some distance posteriorly, but not separated from it and distinguishable only in a few instances; it is to the distal portions of the centrioles that the spindle is connected. In *Spirotrichonympha bispira* a centriole is believed to follow one of the two bands for some distance; it is not separate from the band except in a few instances in division. In mitosis a new flagellar band, with a centriole that follows it closely or may be partly separate from it, grows to a certain length, detaches and migrates to another part of the cell. As it migrates a spindle forms between the two centrioles. In *Spirotrichonympha* the attachment of the rather broad spindle is near the base of the region where the spirals are particularly small and close together—a region that may be compared to the rostrum.

In the above-mentioned hypermastigotes there is bilateral equality—modified in some species by spiraling—in the arrangement of the structures considered to be centrioles. In the interphase two elongate centrioles of equal size are described, or both are unrecognizable (except, often, for their proximal ends) but appear sym-

metrically at the onset of mitosis. Exceptions to this bilateral equality may exist in *Spirotrichonympha bispira* and *Teratonympha mirabilis*. In the former species, Cleveland (1938a), while uncertain about the centrioles in the interphase, admitted three possibilities: persistence of an elongate centriole along one of the flagellar bands, degeneration of all but the anterior part of an elongate centriole, or persistence of one long and one short centriole. In the latter species Cleveland (1938c) found specimens that suggested the existence of one long and one short centriole in the interphase.

In contrast to the usual situation in these flagellates is that found in the species of *Trichonympha* in *Cryptocercus punctulatus*, according to Cleveland *et al.* (1934, text fig. 32). The two centrioles are represented by a pair of crescentic bodies at the anterior end of the rostral tube, and one of them is extended in a rod in the center of the rostral tube. Bilateral equality in their arrangement does not exist except in mitosis, when the other crescentic body is extended in a rod; and then not at the beginning of mitosis.

In the species of *Trichonympha* that I have seen in termites, bilateral equality in the arrangement of rostral structures does exist. The crescentic bodies are each at the anterior end of a half of the rostral tube, but neither of them is extended in a rod. The central rod in the rostral tube of *T. turkestanica* is no more visibly connected to one of these than to the other. It is exactly axial in position, and, having no relation at any time to the poles of the achromatic figure, it cannot be regarded as a centriole.

In the species of *Trichonympha* in which the poles of the spindle are in the region of the posterior ends of the rostral tube halves (*T. turkestanica*, *T. campanula*, *T. collaris*, *T. sphaerica*, *T. agilis*) the position of the achromatic figure corresponds to that eventually reached by the species in *Cryptocercus*. In the roach *Trichonymphas* the elongate centrioles, in all except the first phases of mitosis, are considered to parallel and to be the same length as the rostral tube halves; and the poles of the spindle, connected to the distal ends of these rods, thus coincide in position with the posterior ends of the rostral tube halves. It may be thought likely that there is an elongate centriole similarly paralleling each half of the rostral tube in the *T. turkestanica* group of species in termites, but I have not been able to see it. Nor does it seem reasonable to regard the rostral tube half itself as a centriole or centrolepharoplast.

Neither the original rostral tube half, nor any structure assumed to parallel it, can be regarded as a division center in the group of species of *Trichonympha* of termites in which the pole of the spindle is not associated with its posterior end (*T. chattoni*, *T. peplophora*, *T. ampla*, *T. lighti*). The relationship in these species is to the new rostral tube half, but the connection is not direct, and the relationship is not to the posterior end. It is possible that a rod comparable to a centriole in its relation to the spindle poles is present along the anterior part of this outgrowth. No such rod has been seen in *T. chattoni*, if actually present it was not demonstrated in the material. It may possibly exist along the new rostral lamella in *T. peplophora*. A rod, connected to the spindle pole, may be present but separate from the new rostral tube half in *T. ampla*; but unfortunately, adequate study of the situation was impossible. Detailed study of division in that relatively large flagellate should throw much light on the division process in *Trichonympha*.

The suggestions made in 1926 by Belar, whose point of view was achieved after extensive consideration of comparative cytology, are of value in interpretation of the division center of *Trichonympha*. His concept is weakened, however, by the fact that at that time relatively little was known of the division process in hypermast-

gotes. In Belar's opinion the division center in *Trichonympha* may be assumed to be centrosome-like and situated at the base of the rostrum, but it is not recognizable as a morphological element. In *Teratonympha* and *Pseudotrichonympha* (mistakenly named *Holomastigotoides*) he designated the central spheres at the ends of the spindle, separate from but connected by filaments to the base of the rostrum. The central spheres in these flagellates are not recognizable in the interphase. Cleveland (1935, 1938c) regarded the connecting filaments as the centrioles, and supposed that the proximal part of them persists in the interphase applied to the inside of each half of the rostral tube. Belar however, though aware of the relationship of the filaments, and engaged in a survey of the division process in Protista in general, expressed no opinion that they might be homologous with centrioles.

In the case of *Trichonympha*, it seems that Belar's concept of a physiological center of formation of the achromatic figure, not permanently defined morphologically, may facilitate understanding of the situation. In the different species of *Trichonympha* it seems that this division center is not always associated with the same structure. Its association with the base of the rostral tube seems in most general agreement with the situation in other hypermastigotes. But it may instead become located in the region of the new outgrowing rostral tube halves, or, as it appears in the *Trichonymphas* of *Cryptocercus*, may be related to still other structures. Thus, in species of the genus circumstances are different. Assuming the existence of a physiological center of division associated with certain diverse structures, may relieve us of the necessity of seeking in each of those structures a homologue of the classic centriole. But it does not further the formulation of a generalized concept of the division process in this group of flagellate protozoa with an extra-nuclear spindle.

### STRUCTURE OF THE NUCLEUS OF TRICHONYMPHA

As a basis for an understanding of the nuclear parasites, it is desirable to give general consideration first of all to the nuclear structure of *Trichonympha*. This description is necessarily based upon fixed material, but there is reason for believing that, except for possible shrinkage of the chromatin mass, there is no great discrepancy from the normal condition.

Detailed descriptions of *Trichonympha* nuclei have been given by Kofoed and Swezy (1919a) in *Trichonympha campanula*, Kirby (1932) in various species from termites, and Cleveland *et al.* (1934) in species from *Cryptocercus punctulatus*. The descriptions and figures in these papers will give information concerning the structure of non-parasitized nuclei; and the first and last named papers show the internal changes undergone in the division process.

The nuclear membrane is well defined and evidently persists throughout the division process. Invasion of the nucleus by parasites must take place by penetration of this apparently relatively firm structure.

Chromatin is abundant in the nucleus of *Trichonympha*. Its arrangement differs to some extent in different species; and it is not exactly the same in the nuclei of all non-dividing specimens within a species. The latter differences probably could be arranged in a chronological series, representing a course of events in nuclear life history; but there is no need, for the purpose of this paper, of attempting to make that arrangement.

Various types of nuclei are represented on one plate in my monograph on *Trichonympha* (Kirby, 1932, pl. 30). Chromatin granules dispersed in isolated, grouped, or linear arrangement are characteristic of some species. Arrangement in coiled strands is more usual, and is the type in most of the parasitized species dis-

cussed in this paper (pl. 12, fig. 4). It is also the type in the species of *Trichonympha* in *Cryptocercus*. Cleveland *et al.* (1934) regarded the strands as prochromosomes; and it is likely that they correspond in number and integrity to the chromosomes. They are interconnected by fibrils. The strands are more or less uneven in outline and variable in thickness. Most frequently they form a compact mass within which they are uniformly distributed. In some nuclei the strands are situated mainly or entirely at the periphery of the central mass; the interior is then occupied by a granular and reticulo-fibrillar matrix (pl. 12, fig. 5). In a given species the nuclei with peripheral chromatin strands tend to be larger than those in which the chromatin mass is compact.

Within nuclei of most species of *Trichonympha* there is a nucleolus-like body situated, usually, at or near the edge of the chromatin mass. I previously reported (1932) that I had failed to find this structure in nuclei of *T. collaris*, *T. sphaerica*, and *T. lighti*. In a diagrammatic figure of *T. grandis* of *Cryptocercus punctulatus*, Cleveland *et al.* (1934) represented a large plastin nucleolus and a smaller chromatin nucleolus. The staining reaction of the structure in *Trichonymphas* of termites is not inconsistent with its being regarded as a plastin nucleolus, though often some chromatic constituents are present. In *T. turkestanica* I have observed two bodies (pl. 21, fig. 116) differing in form, position, and staining reaction. The spheroidal, peripheral body is a plastin nucleolus. The elongated, often curved one (pl. 21, fig. 117) corresponds to the chromatin nucleolus of Cleveland; and the structure of similar shape and position in *T. campanula* is evidently of the same nature (p. 193). The plastin type of nucleolus, present in most species of *Trichonympha*, seems to be absent from *T. campanula*, as from other species in *Zoötermopsis*.

Most often the nucleolus has a peripheral position, being situated in a clear area excavated in one side of the chromatin mass (pl. 12, fig. 4). Sometimes, however, it is within the mass, though usually closer to one side than to the center (pl. 12, fig. 5). When the chromatin mass is contracted, so that there is a wide clear area between it and the membrane, the nucleolus is sometimes left free of the mass within that clear area. And in instances of extreme contraction, so that the chromatin lies in a lateral mass more or less crescentic in optical section, the nucleolus may lie apart from it in the remaining space.

Particular attention has been paid to structural detail and variability in the nucleolus of *Trichonympha chattoni* from *Glyptotermes montanus* (pl. 12, figs. 6-16). It has been necessary to study this variability in order to avoid confusion with early invasion stages of nuclear parasites. The material was fixed in Champy's fluid or Schaudinn's fluid, and stained in Heidenhain's iron-haematoxylin or Delafield's haematoxylin. The structural detail of the nucleolus appeared the same in all preparations, except for intensity and color of stain. Since, with few exceptions, the nucleoli of species of *Trichonympha* have been observed to be homogeneous or vaguely vacuolated, the structure of that of the flagellates from *G. montanus* is of particular interest.

The shape of the nucleolus in this species is spherical or spheroidal, and its diameter most frequently is about  $2\mu$ . In some instances the size is larger, reaching  $4\mu$  or more. The largest nucleoli, with a diameter of about  $6\mu$ , have been found in nuclei that appear abnormal in the distribution of chromatin, with the mass contracted to one side (pl. 12, fig. 13).

The structure varies in both small and large nucleoli. In some instances it appears solid and compact (pl. 12, fig. 7), but in most it is vesicular (pl. 12, fig. 8). The smallest nucleoli usually show a distinct membrane and in the center a large granule, which generally is about half the diameter of the nucleolus. The granule

varies in relative size, however; and when the nucleolus is larger there often are two granules (pl. 12, fig. 13), or one main granule and a number of others (pl. 12, fig. 15). In one specimen a much enlarged nucleolus, measuring  $7\mu$ , contained an elongated curved body stained deeply with Delafield's haematoxylin after Schaudinn's fluid, in the same manner as the granules (pl. 12, fig. 14).

Enlargement of the plastin nucleolus of *Barbulanympha* was described by Cleveland *et al.* (1934). The manner in which it takes place is not strictly comparable with what has been described above, because it is related to a definite phase in life history both of the hypermastigote and of *Cryptocercus*. The nucleolar enlargement takes place in both non-dividing and dividing *Barbulanympha*, in the days before and after ecdysis of the roach. It is possible, however, that the instances described by Cleveland of extreme enlargement of the nucleolus, causing nuclear hypertrophy and pushing the chromatin to the periphery, are actually instances of parasitization. There is a suggestive similarity between his figures 87-88, plate 18, in which the nucleus of *Barbulanympha* is nearly filled by the supposed enlarged plastin nucleolus, and figure 75, plate 16 in this paper in which the nucleus of *Trichonympha corbula* is parasitized by *Caryolettira anulata*.

Besides the chromatin substance and the nucleolus, the fixed nucleus of *Trichonympha* shows numerous minute granules. These are often arranged in a peripheral granular zone (pl. 12, figs. 1, 4), as was noted by Kofoid and Swezy (1919a) in *Trichonympha campanula*. This granular zone is not always evident, however; it is of course not evident when the chromatin extends to the membrane. The fine granules are then dispersed among the nuclear contents; or, if the chromatin is peripheral, are in the central granular and reticulo-fibrillar mass (pl. 12, fig. 5). When the chromatin is abnormally contracted to one side, the rest of the nucleus is occupied by fine granules (pl. 12, figs. 13, 14). The presence of these granules should be noted in connection with the occurrence of parasites, which must be distinguished from them.

In the nucleus of *Trichonympha campanula*, Kofoid and Swezy described a zone of large, clear alveoli between the outer granular area and the central chromatin mass. This region is not now regarded as actually an alveolar layer (Kirby, 1932), but the fibrils that extend from the chromatin to the membrane (pl. 13, figs. 17, 18) may suggest such a structure. Cleveland *et al.* observed, in *Trichonympha* of *Cryptocercus*, fibrils lying between the chromatin bodies and the membrane as well as interconnecting the chromatin bodies. These fibrils are a general characteristic of nuclei, not only of *Trichonympha* but of many protozoa; but they do not in this hypermastigote constitute a distinct zone. Often the chromatin is so distributed that the region is not apparent.

#### PARASITIZATION OF THE NUCLEUS OF TRICHONYMPHA

Before considering in detail the characteristics of the several kinds of parasite, it is apposite to make a general survey of the occurrence of nuclear parasites in the material studied. The most useful preparations were those of *Trichonympha corbula* from *Procryptotermes* sp. of Madagascar; in certain series of slides the majority of nuclei were parasitized. It is largely because of this material that I have undertaken to report on nuclear parasitism in *Trichonympha*. In most preparations parasitized nuclei were encountered only occasionally, if at all, generally with some slides showing none and others not more than a half dozen that could readily be recognized. A higher incidence than usual, though much less than in the Madagascar *Procryptotermes*, was found in *Neotermes howa* and *Glyptotermes montanus*. On the whole, it appears that nuclear parasitism is widespread among

species of *Trichonympha*; but in a population it is likely to occur in a small incidence. There may be a flare of disease, however, in which a large percentage of nuclei are invaded, as in the Madagascar *Procryptotermes*.

In the preceding paragraph I have written of nuclear parasitism without reference to the type of parasite. Inspection of the figures on plates 13-19 makes it evident at once that there is considerable variety in the appearance of the organisms in parasitized nuclei. The material studied has not been adequate to provide a certain basis for sorting all these structures into unequivocal systematic categories, or arranging them positively in life-history sequence within the categories. One can distinguish however, several forms that apparently are Micrococcaceae, and larger organisms that show certain similarities to some Chytridiales and Haplosporidia.

Is there not, one may ask, an alternative interpretation of these granules which I have considered to be cocci? Why may they not be the result of alteration of nuclear structure by various circumstances in the process of preparing the material? Or, perhaps, they are the consequence of degenerative or other changes in the nucleus prior to fixation? My observations, made with these possibilities in mind, failed to convince me that the granules could be regarded as anything else than microorganisms. The state of the cytosome in the parasitized flagellates served as an indication of the condition of the preparations.

I have studied a large number of nuclei of *Trichonympha*, in material that had been treated in the same way as the preparations in which the granules were found, and in them no such structures were present. The morphological characters of the granules, which—all species taken together—range from less than  $\frac{1}{2}\mu$  to more than  $1\frac{1}{2}\mu$  in diameter, shows very considerable constancy, a fact that can hardly be harmonized with the view that they are degeneratively altered nuclear substance. In showing forms that may be taken to indicate binary fission, they answer a requirement in the assignment I have made. Where there is hypertrophy of the granule-filled nucleolus and nucleus, and disappearance of the chromatin, no other interpretation of their nature seems possible; and the granules in such hypertrophied organelles correspond to those observed in other circumstances.

Before an organism is given a systematic name, it is desirable that it be known well enough so that there is no question concerning its separation from other forms that may occur in the same situation. One should also make certain, so far as possible, that the name is being given to a systematic unit, and not to a group from which such units will later be separated. It has been impossible to achieve this degree of certainty with respect to the taxonomy of the nuclear parasites of *Trichonympha* here described. It has therefore been a problem whether to give names to them, or merely to describe them as completely as possible, leaving the problem of nomenclature to be resolved later, if at all. It has seemed to me desirable to adopt the former alternative. The likelihood that an opportunity will arise in the near future for clarification of the nomenclatural problems is remote, because of the inaccessibility of many of the sources of material. Specific designation has the advantage of facilitating reference and providing a definite status in scientific literature. Furthermore, the greater number of similar parasites, many of which have been named, are known no better than, or not so well as these organisms from the nucleus of *Trichonympha*. The specialized type of habitat renders it extremely unlikely that they belong to any already designated species.



NUCLEAR PARASITES ALLOCATED TO THE GENUS *CARYOCOCCUS*  
DANGEARD

It is impossible, on the basis of the observations reported below, to give complete diagnosis of the nuclear parasites that I have placed in the genus *Caryococcus*. Nevertheless, the known characteristics of the microorganisms, in morphology and effect on the nucleus, are sufficient to define them in reasonable degree. An adequate diagnosis, sufficient to be a basis for comparison with known bacterial species, would have to include data of a type that I have not found it possible to obtain. Allocation to the genus *Caryococcus* is a matter of convenience.

The genus was established by Dangeard (1902) for a parasite of the nucleus of *Euglena deses*, named *Caryococcus hypertrophicus*. The nucleus of the infected flagellates became hypertrophied to as much as two-thirds of the volume of the cell. The euglenas became colorless, and reddish granules, apparently residues, accumulated in the cytoplasm. Movement continued for many weeks, but the flagellates did not divide. In the early stage of infection, the nucleolus was replaced by a vacuole containing corpuscles. The chromatin mass became reticulated and restricted to the periphery of the nucleus. The interior of the parasitized nucleus was divided into irregular compartments, limited by trabeculae of chromatic substance. The compartments were filled with densely packed, rounded corpuscles.

***Caryococcus nucleophagus* sp. nov.**

(Pl. 13, figs. 17-31; pl. 14, figs. 32-35.)

*Diagnosis*.—Spherules with a diameter of about  $\frac{1}{2}\mu$ , sometimes arranged in pairs, sometimes with a thicker, crescentic, stainable area at the periphery on one side; parasitic within the nucleus, exterior or interior to the chromatin mass, which may be diminished in amount, but does not disappear, nor is the parasitized nucleus appreciably hypertrophied.

*Hosts*.—*Trichonympha corbula* Kirby of

*Procryptotermes* sp. nov. Madagascar. T-4374. (Slides TP-3137:19, 17, 3.)

*Kaloterme* (s.l.) *castaneiceps* Sjöstedt. Madagascar. T-4505. (Slides TP-3191:3, 1.)

*Kaloterme* (s.l.) sp. nov. Madagascar. T-4421. (Slides TP-2160:2.)

*Kaloterme* (s.l.) *longus* Holmgren. Madagascar. T-4442. Slides TP-3163:4.

Nuclei of *Trichonympha corbula* from the species of *Procryptotermes* were extensively parasitized by this microorganism. The material examined was derived from five colonies of termites: TP-3134 from T-4359, 11 km. east of Mahabo; TP-3137 from T-4374, 8 km. south of Mahabo; TP-3139 from T-4378, 37 km. south of Mahabo; TP-3149 from T-4400, Ankazoabo; TP-3094, 3103 from T-4307, 20 km. n.e. of Maevatanana.

In the first four series listed, a large proportion of the nuclei were parasitized. The incidence was particularly high in some slides of series 3134 and 3137, where the incidence of infection of nuclei of *Trichonympha* exceeded 90 per cent. The parasite described here was not found at all in nuclei of *T. corbula* series 3094 and 3103; in those series a different microorganism was present in many specimens.

There is no marked hypertrophy of nuclei invaded by *Caryococcus nucleophagus*. Though many parasitized nuclei are a little larger than some that are not parasitized, there are unparasitized nuclei as large as any that are parasitized.

In the distribution of the microorganisms there are two general types, both of which are frequent. In one type (pl. 13, fig. 19) they are exterior to the chromatin mass; in the other (pl. 13, fig. 26) within it. In the former arrangement, the chromatin mass is compact, leaving a space between itself and the membrane; and in this space microorganisms occur. They are often distributed rather evenly. Sometimes they are massed at one side; and opposite the group of parasites, which may

be closely packed together, there is often an indentation in the chromatin mass (pl. 13, fig. 20). Frequently the group extends partly within that mass. In spite of being closely packed, the granules appear clearly to be isolated bodies.

The presence of the parasites within the chromatin mass characterized the majority of *Trichonympha* nuclei on slides of series TP-3137. In these the chromatin was restricted to the periphery of the nucleus, with occasional strands extended inward. In no instance was chromatin absent, and often it appeared not to be greatly diminished in total amount, in spite of the hollowing out of the interior of the mass. In some nuclei the microorganisms were located both within and exterior to the chromatin mass (pl. 13, figs. 20-21). On other series from *Procrptotermes* there were some nuclei with the parasites in each position. The parasites are capable of developing in either or both situations. Although in some individual termites there may be a tendency for most of the parasites to be exterior, whereas in others they are interior, it seems clear that the same organism is involved.

The parasites, with a diameter of about  $\frac{1}{2}\mu$ , show a considerable degree of constancy in size in the infections of the Madagascar *Procrptotermes*. Often they are arranged in pairs (pl. 13, fig. 18), and many dumbbell forms, indicating the process of binary fission, have been observed (pl. 13, figs. 24, 25). In some preparations, the granules are differentiated to show a deep-stained rim, which is thicker on one side than on the other (pl. 13, figs. 23, 27).

On several slides a number of specimens of *Trichonympha corbula* were found with two nuclei, several with three, two with four, and one (pl. 14, fig. 32) with six. In some instances, where there were two nuclei, one nucleus was in the usual position, associated with the parabasal bodies and the other was separate; in others, particularly with the larger numbers of nuclei, all of them were displaced, often into the posterior part of the body. When there were several nuclei, all were somewhat smaller than normal. The mitotic processes that give rise to such extra nuclei, particularly the odd number of three, are not known in *Trichonympha*. It is not unlikely, however, that a multipolar division figure existed, such as Cleveland (1938) reported in *Barbulanympha*; and because of the irregularity division of the cytosome failed to take place. The point of special interest for this investigation is that all the nuclei contained masses of the bacterial parasites within the chromatin mass, or infrequently exterior to it. Usually the position of the parasites was the same in all nuclei of a specimen; but in one instance, with two nuclei, the granules were exterior to the chromatin in one nucleus and interior in the other. On these slides, more than 90 per cent of all the *Trichonympha* nuclei were parasitized.

Parasites apparently identical with those described above from *Trichonympha corbula* of *Procrptotermes* have been observed in *T. corbula* of some other Madagascar termites. On slides from a colony of *Kal. (s.l.) castaneiceps* (T-4505), granules about  $\frac{1}{2}\mu$ , occasionally up to  $\frac{3}{4}\mu$  in diameter were found in all but a few *T. corbula* nuclei (pl. 14, figs. 33-34). When suitably differentiated, they showed a crescentic area at one side. In all these nuclei the chromatin mass was contracted, and the granules, ranging in number from very few to many, were exterior to it. Normal nucleoli were often present along with the parasites; but in several instances the nucleolus had been invaded by the parasites and had been greatly enlarged (pl. 14, fig. 34). There was no particular enlargement of any of the parasitized nuclei.

Preparations from another colony of *Kal. (s.l.) castaneiceps* (T-4476) had none of these microorganisms. Preparations from one colony (T-4421) of a termite classed by Emerson as *Kal. (s.l.)* sp. nov. near *castaneiceps* had some *T. corbula*

nuclei with a small number of parasites exterior to the chromatin mass (pl. 14, fig. 35); in those from another colony of the same species (T-4468) no parasites were found. In *Kal. (s.l.) longus* one series (TP-3163) had the parasites in most of the nuclei of *T. corbula*, whereas in other series only a few were present, or they were altogether absent.

***Caryococcus invadens* sp. nov.**

(Pl. 14, figs. 36-39)

*Diagnosis*.—Spherules 1-1.5 $\mu$  in diameter, sometimes arranged in pairs, often internally differentiated with stainable central or peripheral granules or stained areas; parasitic in the nucleolus and nucleus; parasitized nucleolus becoming greatly enlarged and crossed by trabeculae, eventually consumed; nucleus becoming moderately enlarged, but chromatin not disappearing.

*Host*.—*Trichonympha peplophora* Kirby

*Neotermes howa* (Wasmann). Madagascar. T-4446. (Slides TP-3175:2, 6, 35.)

In a few nuclei of *Trichonympha peplophora* from *Neotermes howa* parasites were observed that resembled *Caryococcus nucleophagus* except for their considerably larger size. The incidence of parasitization was exceedingly low, in no way comparable even to the more infrequent infections with the smaller coccus in its hosts.

The more heavily parasitized nuclei were somewhat, but not excessively, enlarged. One measured 15 $\mu$   $\times$  24 $\mu$ , another 25 $\mu$   $\times$  21 $\mu$ ; the normal nuclear diameter is 11-15 $\mu$ . When many parasites were present, the chromatin mass was much altered, but the amount of chromatin was not greatly diminished.

In all instances the parasites were present in the nucleolus also. One specimen, with a scarcely altered chromatin reticulum, had an enlarged nucleolus within which were a number of spherules corresponding to the parasite in size, but no parasites were present elsewhere. In the other nuclei, parasites were present in the enlarged nucleolus and elsewhere (pl. 14, figs. 36, 37). The parasitized nucleolus, whose diameter in several instances was 7-8 $\mu$ , may have a rather complex appearance in early stages. Not only are the several parasite spherules present, but deep-staining strands or trabeculae occur in the nucleolar substance; granules of nucleolar origin may also occur (pl. 14, figs. 37, 38). It appears that later the parasites consume all the nucleolar substance; and after the nucleolus is obliterated, they presumably enter the group of parasites outside. Plate 14, figure 39 represents a body of parasites, among others, that had evidently originated in the nucleolus; but the only evidence of that structure was the form of the mass and the surrounding vestiges of chromatin.

The individual parasites are spherical in form, 1-1.5 $\mu$  in diameter. They may be arranged in pairs or not. Many show a dumbbell form (pl. 14, figs. 36, 39), and some, in a late stage of binary fission, are connected by only a narrow bridge. In most preparations they appeared homogeneous, but occasionally showed structural detail (pl. 14, fig. 37). In this figure, some appear double, deeply stained except for an equatorial clear band; others show deeper staining at one side. In many there is a granule in the center, and often there are a number of peripheral granules. Each body or pair of bodies is surrounded by and set apart from the alveolar matrix of the nucleus by a clear space.

***Caryococcus dilatator* sp. nov.**

(Pl. 14, figs. 40-43; pl. 19, figs. 92-93)

*Diagnosis*.—Spherules  $\frac{1}{2}\mu$  or less in diameter, internally differentiated with stainable granule or area peripherally situated; parasitic in nucleus and nucleolus; nucleus becomes greatly enlarged and the chromatin mostly or entirely disappears.

## Hosts.—

*Trichonympha chattoni* Duboseq and Grassé.

*Glyptotermes iridipennis* Frogg. Australia. T-304. (Slide TP-309:52.)

*Kalotermeis schwartzi* Banks. Florida. T-4615. (Slide TP-3295:19.)

*Trichonympha peplophora* Kirby

*Neotermes howa* (Wasm.) Madagascar. T-3187. (Slide TP-3187:34.)

*Trichonympha saepicula* Kirby

*Eugitermes kirbyi* Snyder. Costa Rica. T-148. (Slide TP-92:3.)

*Trichonympha tabogae* Kirby

*Kalotermeis tabogae* Snyder. Panama. T-227. (Slide TP-136:32.)

*Trichonympha teres* Kirby

*Neotermes mervensis* Sjöstedt. Tanganyika. T-2009. (Slides TP-1089:13; TP-1081:13.)

*Trichonympha turkestanica* Bernstein.

*Anacanthotermes turkestanicus* Burm. Egypt. T-1000. (Slide TP-1005:1.)

† *Anacanthotermes vagans* Hagen. Arabian Peninsula.

The bacterial parasites included under the name *Caryococcus dilatator* individually resemble *C. nucleophagus*. They are  $\frac{1}{2}\mu$  or less in diameter, and when suitably differentiated sometimes show a deep-staining peripheral region thicker on one side. But there are differences in the development of the organism and its effect on the nucleus, such that it seems advisable to give it taxonomic distinction.

The record from *T. turkestanica* of *Anacanthotermes vagans* is based on the study of alcoholic specimens, but the parasite seemed to be the same as in the other material. In all material the incidence was low, only certain slides showing the organism at all, and usually less than a dozen *Trichonymphas* on a slide having it in sufficient abundance to be readily recognized.

Enlargement of the nucleus is great. In *Trichonympha peplophora* of *Neotermes howa*, a nuclear diameter of  $30\mu$  was attained, in *Trichonympha chattoni* of *Glyptotermes iridipennis*,  $20\mu$ ; the normal diameter in these two hypermastigotes is  $11-15\mu$  and  $8-13\mu$ , respectively. The nucleus retains its normal position in the body. Those that are heavily parasitized can be readily recognized by the enlargement and also by their dense appearance. In many preparations they appear so densely stained that no detail can be made out.

In earlier stages of parasitization the chromatin mass is compacted in the center, but is not greatly diminished in amount. The small parasites are abundant at the periphery, with a clear space between them and the chromatin. The nucleolus may also be invaded, and consequently enlarged. Figure 41, plate 14, represents a nucleus of *Trichonympha peplophora* whose nucleolus is invaded by a dense mass of minute parasites. Similar parasites are present in the peripheral region of the nucleus; nuclei and nucleoli of *T. saepicula* have been observed to be similarly parasitized (pl. 19, fig. 92). The prophase nucleus of *T. peplophora*, with paired chromatids (pl. 14, fig. 40), has no parasites except in one rounded mass. These are notably larger than the others, having a diameter of about  $\frac{1}{2}\mu$ . The nucleus, having entered upon a normal prophase, had not been injured, so the invasion must have been very recent. The situation suggests that this invasion occurred in the nucleolus, and that the rounded mass represents that structure. There is no other nucleolus, and in nuclei at a similar stage of development in *Trichonympha chattoni* (pl. 12, figs. 15, 16) nucleoli have often, though not always, been recognized.

In later stages of parasitization the enlarged nucleus is filled with a dense mass of granules, and the chromatin has greatly diminished in amount. The position of the chromatin vestiges varies. In a nucleus of *Trichonympha peplophora* (pl. 14, fig. 42) what is left of the chromatin is compacted in a central spherule  $5-6\mu$  in diameter. In other nuclei of *T. peplophora*, and those shown from *T. chattoni* (pl. 14, fig. 43) and *T. turkestanica* (pl. 19, fig. 93), the chromatin is in strands crossing the interior among the granules, and some also is peripheral.

On slides from *Neotermes meruensis*, no parasites other than that described here as *Caryococcus dilatator* were observed in nuclei of *Trichonympha teres*.

### ***Caryococcus cretus* sp. nov.**

(Pl. 14, fig. 44; pl. 15, figs. 45-46)

*Diagnosis*.—Spherules 1-1.5 $\mu$  or more in diameter, in preparations appearing clear with usually a chromatic, sharply defined crescentic structure peripherally or interiorly situated, sometimes with two such bodies or several chromatic granules; parasitic in nucleus, parasitized nucleus enlarged only moderately or not at all, chromatin altered but not greatly diminished in amount.

*Host*.—*Trichonympha corbula* Kirby.

*Procryptotermes* sp. nov. Madagascar. T-4807. (Slides TP-3094:11, 17.)

*Caryococcus cretus* has been found in *Trichonympha corbula* from only one colony of several collected. In this material, *Caryococcus nucleophagus*, which was more or less abundant in four other colonies, did not occur. *C. cretus* was present in a large proportion of the nuclei of the flagellate, but not in all. In most nuclei, the spherules were not numerous. As in *C. nucleophagus*, some were exterior to a contracted chromatin mass (pl. 14, fig. 44), some interior to a peripheral chromatin reticulum (pl. 15, fig. 46). The more heavily parasitized nuclei were somewhat enlarged, but not to a degree comparable to the enlargement caused by *Caryococcus dilatator*.

Individual spherules have a diameter of 1-1.5 $\mu$  or more, about twice the size of those of *C. nucleophagus*. In addition to this difference in size the internal structure is such as to warrant taxonomic distinction from *C. nucleophagus*. In all preparations the parasites appeared vesicular; whatever the stain, among those used, they are rather clear except for some deep-staining structure. In similar preparations, *C. nucleophagus* generally appeared altogether rather deep stained and homogeneous. In the vesicle, a deep-stained crescentic structure is present at one side or in the interior (pl. 14, fig. 44). This is not comparable to the crescentic peripheral area that sometimes appears in *C. nucleophagus*. The latter structure appears as part of the wall, merging into other parts; whereas the former appears as a separate, isolated structure. Usually this crescentic body is single in the vesicle, but sometimes there are two such bodies, or a few large granules are present (pl. 15, figs. 45, 46).

When present within the reticulum of chromatin, the vesicles are dispersed in the finely granular nuclear matrix. They are not gathered in a close-packed group, as is frequently characteristic of the other species.

Nucleoli of normal appearance have been seen in parasitized nuclei, but in no instance has a nucleolus been observed to be invaded by the parasite.

### **Nucleophaga-like parasites**

(Pl. 15, figs. 47-59)

A fairly numerous parasite has been found in nuclei of *Trichonympha chattoni* in a series of slides from *Glyptotermes montanus* of Java. On some slides the incidence of infection was about 10 per cent. In its mature state the parasite resembles *Nucleophaga*, but in the earlier phases of its development it exhibits certain differences from the life cycle of that intranuclear chytrid as usually understood.

In figure 47, plate 15, there are two bodies that appear like poorly prepared small amoebae. They are irregular in outline, vacuolated in internal structure, are relatively large—having a diameter of 7 $\mu$ ,—and occupy a somewhat enlarged nucleus, in which the chromatin is in peripheral masses. In another nucleus there was seen one amoeboid body 6  $\times$  6.5 $\mu$ , and two smaller ones of 5 $\mu$  connected in dumbbell form.

In several nuclei, particularly on certain slides, a larger number (6–10) of similar irregular, vacuolated bodies were found. These parasitized nuclei were in the usual position, and were not greatly enlarged. The individual parasites were smaller than when there were only 2 or 3, the usual diameter being 3 or 4 $\mu$ . Those in figure 48, plate 15, have a diameter of 2.5–4.4 $\mu$ . Some of them are in dumbbell form, as though in binary fission, but the somewhat imperfect character of the preparations made it impossible to be certain of this. The chromatin in nuclei parasitized in this manner was somewhat reduced in amount and arranged in a peripheral mass or in a ring. The parasites often caused protrusion of the membrane, giving the nucleus an irregular form.

A large number of nuclei containing numerous spheroidal bodies have been found. Generally all the spherules in a given nucleus are rather uniform in diameter, but the size varies in different nuclei. In some they have a diameter of 2 $\mu$ ; more often they measure 3–4 $\mu$ . The spherules show no structural detail. In Schaudinn-fixed, Heidenhain-stained material suitably differentiated for normal nuclei of *Trichonympha* they often are homogeneous and dark gray. In Delafield-stained preparations, also after Schaudinn's fluid, they often appear somewhat vacuolated (pl. 15, fig. 49). When Delafield stain is particularly intense, they take a rather deep, uniform stain.

Two-thirds or more of the parasitized nuclei are almost completely occupied by spherules of this type. The chromatin is reduced in amount, but vestiges are present in varied arrangement. Sometimes the chromatin is displaced in a crescentic peripheral zone; sometimes it is in more evenly distributed peripheral strands and granules (pl. 15, fig. 50). Often it is not restricted altogether to the periphery, but strands and granules are present among the spherules.

Nucleoli of the vesicular type described earlier in this article as characteristic of my preparations of *Trichonympha chattoni* from *Glyptotermes montanus* have been seen in many parasitized nuclei. The nucleolus is situated peripheral to the group of spherules, and is not enlarged. This parasite does not invade the nucleolus; which is present along with the parasite until the late stages of development; then it disappears.

Nuclei parasitized as described above are enlarged from very little to twice the normal diameter of 10–12 $\mu$ . In some instances they are in the position normal to the *Trichonympha* nucleus; but often, particularly when the enlargement is greater, they are displaced into the posterior cytoplasm. Figure 57, plate 15, shows the nucleus ruptured, and with some of the spherules scattered in the cytoplasm. As the nucleus is in place and only moderately enlarged, and as the spherules (in Heidenhain-stained material) are homogeneous except for irregular vacuolization, it seems likely that the nucleus has been ruptured prematurely or accidentally. Several other ruptured nuclei have been observed in which the spherules were homogeneous or simply vacuolated; among them were chromatic rods which escaped with them into the cytoplasm. Most likely the breaking of these nuclei was also the result of accidental damage.

In some parasitized nuclei the spherules contain a laterally situated, deep-staining, nucleuslike granule. The structure of this granule being similar to that of mature spores of parasites of the type of *Nucleophaga*, for instance the *Nucleophaga*-like parasite of *Caduceia theobromae*, it is thought that it represents the mature state of development. The size of the spores is usually 3–4 $\mu$ , but in one instance (pl. 15, fig. 53) they are variable in size, reaching 5.7 $\mu$ , and in another (pl. 15, fig. 52) they are about 2.5 $\mu$ . When they are most numerous the individual size is smallest. The nucleuslike granule is variable in relative size. In bodies of 4 $\mu$ ,

a granule diameter of  $1\mu$  is frequent; but the size may be greater or smaller than this. The granule appears most distinct in Heidenhain-stained material; in Delafield preparations the rest of the substance also stains rather deeply, and the granule is obscure.

Nuclei containing mature spores are generally more greatly enlarged than those with homogeneous or simply vacuolated spherules, and they are usually displaced. When they are spheroidal in form, their diameter is most often a few microns more than 20. Frequently the shape is ellipsoidal, one dimension being about  $25\mu$  and the other a few microns less. The largest seen measured  $21 \times 28\mu$ .

The membrane of parasitized nuclei is always distinct, and is not tenuous even in much enlarged nuclei. In the stages with mature parasites, it is often thickened in places by chromatic material adherent to it. This gives it an irregular outline inwardly, but it may be smooth at intervals or entirely.

A ruptured nucleus with mature spores is represented by figure 56, plate 15. Spores are escaping into the adjacent cytoplasm. The body of the host *Trichonympha* has been ruptured, and traces of it remain. The break of both body and nucleus may have been the consequence of injury in smearing; but there are indications that it occurred naturally. *Trichonymphas* in the smear nearby were not injured, and the nuclear membrane is absent from the vicinity of the region of the break in the nucleus. Such a break in the nucleus must, of course, be a normal phenomenon in development; and it is probable that the flagellate is destroyed at the time.

In a number of termites other than *Glyptotermes montanus*, nuclei of *Trichonympha chattoni* have been found containing stages of parasites similar to the one just described. Whether or not they belong to the same species, however, cannot be decided, because very few stages have been found. In *Glyptotermes iridipennis* several enlarged nuclei of *Trichonympha chattoni* contained spores with a granule at one end. In most of these the granule was very large, half or more the diameter of the spore (pl. 15, fig. 58), but in others (pl. 15, fig. 59) it was small. In the latter specimens, chromatin strands extended among the bodies, whereas in the former there was little or no chromatin left. Similar parasites have been found in *Trichonympha chattoni* from *Glyptotermes brevicaudatus*, and in the same species from *Glyptotermes* sp. nov. from Uganda (T-2090). The very numerous spores of a parasitized nucleus of the latter species had a diameter of  $1.5-2\mu$ . In *Trichonympha quasilla* from *Kalotermes perezi* a *Nucleophaga*-like parasite was observed in both multinucleate plasmodium phase, with numerous deep-stained granules in a common matrix, and in a mature stage. In the latter the nucleus was enlarged to  $32 \times 20\mu$ .

#### **Caryoletira gen. nov.**

*Diagnosis*.—Multinucleate phase, with relatively large compact nuclei, undergoing sporogony to form numerous spores with a usually relatively large granular nucleus toward one end and more or less abundant granules of metachromatic substance elsewhere. Parasitic in nucleus, consuming chromatin. Type species *C. anulata*.

#### **Caryoletira anulata sp. nov.**

(Pl. 16, figs. 60-75; pl. 17, figs. 76-82)

*Diagnosis*.—Multinucleate phase with dense, spheroidal or ellipsoidal nuclei about  $1\frac{1}{2}-2\mu$  in diameter, between them chromatic granules; spores variable in number, up to 200 or more, usually  $2.5-4\mu \times 2-3\mu$ ; nucleus in spore at one end, usually relatively large in size, densely granular; metachromatic substance abundant, granules arranged in an equatorial ring, sometimes filling entire region of half of spore; parasitic in nucleus consuming chromatin and causing considerable enlargement.

*Host*.—*Trichonympha corbula* Kirby

*Procrryptotermes* sp. nov. Madagascar. T-4359. (Slides TP-3134:5, 6, 7.)

I have found *Caryoletira anulata* fairly frequently in *Trichonympha corbula* from *Procryptotermes* collected near Mahabo, Madagascar. The parasite was also present in other series from this termite. In some of the same preparations *Caryococcus nucleophagus* was found as an abundant parasite of the nucleus of *T. corbula*.

The earliest stages of infection have not been found; in the youngest ones encountered, a considerable number of spherules were already present. The nucleus shown in figure 60, plate 16, has a longer diameter of about  $12\mu$ , and the homogeneous spherules measure about  $1\frac{1}{2}\mu$ . Among them are a few deep-staining granules. Although most of the chromatin is in peripheral strands, there are extensions inward among the spherules.

Other nuclei in a similar state of parasitization have been seen. The spherules have a diameter of about  $1\frac{1}{2}\mu$ , and stain pale with Heidenhain's haematoxylin. Among the spherules are small, deep-staining granules. The chromatin is usually in peripheral strands; but in a few instances the chromatin mass is compacted in the central part of the nucleus, and spherules of the same type, with small, deep-staining granules among them, are peripheral to the mass.

Nuclei in the earlier stages of parasitization, described above, are only moderately enlarged; but as the number of spherules increases the nuclei attain more than twice the diameter normal for the species. In these more advanced stages (pl. 16, figs. 62-64), the bodies representing the parasites are often ellipsoidal in form, and measure  $1 \times 1\frac{1}{2}\mu$  or more. Among them, again, are small, deep-staining granules, and there is still considerable chromatin in a peripheral reticulum or strands. Figures 63 and 64, plate 16 show the same nucleus, the former a surface view in which the peripheral chromatin is visible at the surface, the latter an optical section.

In the nuclei described above, the spherical or ellipsoidal bodies that represent the parasites stain pale with haematoxylin, as compared to the chromatin, and they are homogeneous. They neither react to dyes in the manner usual for chromatin, nor show granules—characteristics which would aid in identifying them as nuclei. But if they are not nuclei, neither is there any evidence that they contain nuclei. It has, furthermore, been impossible to decide whether they are embedded in a common cytoplasmic mass or are isolated bodies. Some observations support the latter view, such as the extension of chromatin strands inward among the spherules. Other facts, such as the organization of the bodies into a definitely bounded group, support the former interpretation, which is most probably the correct one.

Nuclei in which the spores have matured may have four times the normal diameter, but there is much variation in the maximum size attained. The form is spheroidal or ellipsoidal. The largest diameter was  $44\mu$ , but one nucleus with mature parasites (pl. 16, fig. 65) measured only  $13\mu$ , which is little more than normal. This small nucleus had remained in the normal position, associated with the parabasal bodies; but usually the nuclei in this stage of parasitization have become displaced. Occasionally in this displacement a part of the nucleus remains in contact with the parabasal bodies (pl. 17, fig. 78), and the result may be a drawing out of this part into a small appendage. That the parabasal bodies adhere firmly to the nuclear membrane is indicated in figure 85, plate 18, a parasitized nucleus of *T. peplophora*. In it there appears to have been some force pushing the nucleus posteriorly, and the membrane is drawn out where parabasal cords are in contact with it.

The number of spores in the small nucleus mentioned above (pl. 16, fig. 65) was only 17, but usually there are 150-200, or more (pl. 16, fig. 74). Chromatin material of the host nucleus may have entirely disappeared, but sometimes there are granules and peripheral strands constituting a greatly reduced amount.



Spores of the parasite are generally ellipsoidal in form and measure  $2.5-4\mu \times 2-3\mu$ . Spores are larger when the number is smaller; the smallest parasitized nucleus (pl. 16, fig. 65) had 17 spores of maximum size. The minimum size of the spores was about  $1.5 \times 1.7\mu$ .

The prepared specimens in which most detail was evident in the spores were those that had been fixed in Hollande's fluid. Schaudinn's fluid and Flemming's fluid appeared to penetrate poorly, and the internal structure was obscure. Spores fixed in those two fluids, when stained in iron-haematoxylin, revealed only a lighter half and a homogeneous darker half. But Hollande-fixed material, stained by Heidenhain technique, showed clear detail.

A constantly occurring structure at one end is probably the nucleus. Typically this is a rounded body, spherical or ellipsoidal in form, and relatively large. It often has a diameter almost equal to the shorter diameter of the spore (pl. 16, figs. 66-70), but in some instances it is considerably smaller than this (pl. 16, figs. 72-73). The spores with a small, compact, nuclear granule bear a closer resemblance to the spores of the *Nucleophaga*-like parasite. In the preparations the nucleus may or may not extend to the periphery. In the latter event there is a clear space between the spore membrane and the nucleus (pl. 16, figs. 65, 66); in the former, the nucleus has a more or less hemispherical form (pl. 16, figs. 68-70).

When too heavily stained, or when not well fixed, the spore nucleus may appear homogeneous (pl. 16, figs. 67-69); but in good, sufficiently well differentiated preparations it is resolved into granules (pl. 16, fig. 66). The nuclear body may be stained heavily, or relatively little, or not at all (pl. 16, fig. 71) in preparations in which in general the haematoxylin stain appears equally intense.

Other granules in the spore stain with iron-haematoxylin either more or less intensely than the nucleus—for the most part more. The nucleus is constant in occurrence and position; but the extranuclear granules vary in occurrence, abundance, and distribution. Often they are arranged in an equatorial ring (pl. 16, figs. 66-70). This ring sometimes appears solid, especially when the nucleus also appears solid. Sometimes there are no granules outside the nucleus except those in this ring, or there are very few (pl. 16, figs. 68, 70). The ring is the most constant of the extranuclear elements. It constitutes the equatorial rim of a hemispherical region opposite the nucleus, and occasionally this hemisphere is a mass of granules. There are all gradations between the condition in which it is a solid mass of granules and that in which only the ring is present. In addition to the ring there may be only a few granules at the periphery of the hemisphere; or these granules may be more abundant and form a peripheral semicircular row at right angles to the plane of the ring (pl. 16, fig. 69). Instead of a semicircular row only, the granules may be more abundant and form a hemispherical layer. This leads to the condition in which the granules fill the area. These cytoplasmic granules are located first at the periphery, and between the group of granules and the margin there is no clear space comparable to that which often surrounds the nuclear body.

As I stated heretofore, in a small percentage of parasitized nuclei the spores of *Caryotetra* contained a small, compact nuclear body (pl. 16, figs. 72-73). Adjacent to this granule was a clear area, then a compact group of granules, corresponding to the hemispherical group, but filling considerably more than half of the spore, and showing no equatorial ring. All spores of this type were of moderate size, the diameter being about  $2.5\mu$ . The differences from the more numerous type suggest a taxonomic distinction; but, from the standpoint of distribution, the occurrence of a second species in only two or three sporangia in a heavy infection of the other type does not seem likely.

In a number of instances nuclei were found ruptured in one place and spores were distributed in the cytoplasm of the flagellate (pl. 17, fig. 77).

Along with the mature spores, a crystalloid body is often present (pl. 16, fig. 74; pl. 17, figs. 79, 81, 82). It was found consistently in both Hollande-fixed and Schaudinn-fixed material from one colony of termites, but only occasionally in material from other colonies, none of it fixed in Hollande. In longitudinal section this body has the shape of a conventional diamond, and in cross section that of a hexagon (pl. 17, fig. 79). It is in form of a hexagonal bipyramid, but in fixed material is often not perfectly regular. It is always located peripherally, and generally produces a protuberance of the nuclear membrane. Figure 80, plate 17, represents the structure in a nucleus in which there are as yet no mature spores, and its size is smaller than is characteristic of nuclei with more advanced parasites. Often, when fully developed, it measures  $5 \times 3.5\mu$ . It is unlikely that this structure is residual chromatin.

A number of instances of parasitization of nuclei of *Trichonympha* by organisms similar to *Caryoletira anulata* have been found in *Kaloterms castaneiceps*, *Kaloterms longus*, and *Neoterms howa*. In *Trichonympha corbula* from *Kaloterms castaneiceps* and *Kaloterms longus* only a few parasitized nuclei, all with matured spores, were found. Variability in size of spores appears in the nucleus represented by figure 83, plate 38, from *Kaloterms longus*; in them the diameter ranges from 2.5 to  $5\mu$ . The situation is different from that in nuclei of *Trichonympha corbula* from *Procryptotermes*; there the spores in a single nucleus have more regularity in size.

Earlier stages of the parasite in *Trichonympha peplophora* of *Neoterms howa* were spherules  $2\mu$  in diameter, among which were chromatic granules and strands. The mature spores were similar to those of *Caryoletira anulata*, with a nuclear mass of granules, often hemispherical in form and separated by a clear space from the spore membrane. The largest spores, reaching  $8 \times 8.5\mu$ , were seen in *Trichonympha peplophora* (pl. 18, fig. 90). The parasitized nucleus shown in figure 86, plate 18, is unusually large ( $59 \times 35\mu$ ) and irregular in form, with numerous protuberances of the membrane; and it contains mature spores varying greatly in size, ranging from 1.7 to  $5.2\mu$ . The nucleus shown in figure 89, plate 18, has been shifted posteriorly from its normal position; but a small part of it, containing one spore, has remained in contact with the parabasal bodies.

No crystalloid body was observed in association with the parasites from *Neoterms howa*, *K. castaneiceps*, or *K. longus*. Considered in connection with the constancy of that body in association with typical *Caryoletira anulata* from *Procryptotermes*, this seems to indicate a physiological difference which may warrant taxonomic distinction. I have not had adequate material, however, to make a well-founded separation of species.

### ***Caryoletira magna* sp. nov.**

(Pl. 19, figs. 96-101)

**Diagnosis.**—Similar to *C. anulata*, but with spherules of early phase of development  $3-3.5\mu$  in diameter, and spores larger, often measuring  $10-12 \times 14-16\mu$  in diameter. Parasitic in nucleus, causing great enlargement.

**Host.**—*Trichonympha turkestanica* Bernstein

*Anacanthotermes ochraeus* Burm. Egypt. T-1007. (Slides TP-1012:18, 19, 25.)

A fairly large number of parasitized nuclei of *Trichonympha turkestanica* have been found. Many of these contained small spherules of the type of *Caryococcus dilatator* (pl. 19, fig. 93). Others contained larger spherules and spores of *Caryoletira*. The material was not so extensive nor so well prepared as that from the

Madagascar *Procryptotermes*; but there seems to be warrant for taxonomic distinction from *Caryoletira anulata*. What, by comparison with *C. anulata*, seems to be an early phase of parasitization by this species, is a group of spherules about  $3\text{--}3.5\mu$  in diameter, enclosed in the chromatin reticulum. In the nuclei represented in figures 96 and 97, plate 17, there are only a few of these. They are more numerous in the nucleus shown in figure 98, plate 19, in which, as has been observed in a number of instances in *T. turkestanica*, the chromatin mass is partly extruded from the nucleus. This extrusion is apparently an abnormal occurrence; but the flagellates had not been mechanically damaged. No instance was observed in which, as in *C. anulata* (pl. 16, figs. 63, 64), the parasites constitute a dense inner mass, whereas the chromatin is all peripheral.

The mature spores reach a large size. In one nucleus, enlarged to  $55\mu$ , they measured  $10\text{--}14\mu$ . The spore shown in figure 101, plate 19, from another nucleus, measured  $11.4 \times 15.4\mu$ . These are very much larger than the spores of *Caryoletira anulata* in *Procryptotermes* sp. The results of fixation were not altogether satisfactory, but there is a clear differentiation of the contents into two regions comparable to those in the other species. The parasites shown in figure 99, plate 19, contain deep-stained strands or rows of granules, segregated in some instances into two groups; and there is a resemblance to mitotic figures. This may represent an earlier phase in development, with division, the spores not being mature. As no other instance of "chromosomes" has been seen, however, I cannot be positive in the interpretation. Each of the mature spores shown in figures 100–101, plate 19, contains at one end a nucleuslike body which is stained deeper than the other material in one instance (fig. 100), and paler in the other (fig. 101). Vacuolated material fills the rest of the spore.

## NUCLEAR PARASITES OF TRICHONYMPHA SAEPICULA

(Pl. 19, fig. 92, 94–95, 102)

In 1932 I mentioned and figured a parasite of the nucleus of *Trichonympha saepicula*. Further search has been made of the slides from the termite host in the hope of learning more about the organism. In the course of that search, a number of nuclei were found parasitized by *Caryococcus dilatator* (pl. 19, fig. 92), and a not inconsiderable number were found with larger parasites.

The published drawing (Kirby, 1932, pl. 26, fig. 33) represents an immature phase in development of the parasite. A still earlier phase was found in the present survey, in a nucleus measuring  $16 \times 12\mu$  and still in the customary position. The chromatin remaining was still fairly large in amount, and located peripherally in a reticulum. The rounded bodies, which were present in numbers in the nucleus, had even outlines, but already showed a coarse vacuolization. As the parasite develops (pl. 19, figs. 94–95) the bodies become more numerous, the chromatin is reduced to only a few granular vestiges, and the nucleus is much hypertrophied. The largest parasitized nucleus was  $31 \times 36\mu$  in size. The rounded bodies representing the parasites enlarge from the size characteristic of the earliest stages found,  $1.5\text{--}2\mu$ . The vacuoles become recognizable as distinctly outlined vesicles. The largest nuclei contain aggregates of vesicles. The aggregates are irregular in form, often measure  $4\text{--}5\mu$  across, and consist of vesicles varying in size from  $1$  to  $3\mu$ . The walls of the individual vesicle are well defined. Where they are contiguous the stain may be deep. The aggregate no longer maintains a smoothly rounded outline. I have seen no structure whatsoever within the vesicles.

In the aggregation of vesicles characteristic of this parasite one is reminded of

the nuclear parasites of *Amoeba viridis* described by Gruber (1904). When the parasites emerged from a ruptured nucleus of *Amoeba*, they were in groups of six granules, and appeared in the form of crosses. The similarity is entirely superficial, however. The parasite of *Trichonympha saepicula* is not *Nucleophaga*, and several observers have identified Gruber's parasite as belonging to that genus.

On some slides of *Trichonympha saepicula* parasites bearing a resemblance to *Caryoletira* were present in enlarged nuclei. Because of the condition of the preparations, a detailed comparison with the other described species is not possible. The nucleus represented by figure 102, plate 19 was displaced from position and measured  $35 \times 31\mu$ . It is filled with spores  $6-9\mu$  in diameter. Each spore has a distinct membrane, and at one end is a large, rounded body that has remained unstained in the iron-haematoxylin preparation. It is recognizable chiefly by its membranous boundary. It is possible that this is the nucleus, but that, as noted in some preparations of *Caryoletira*, it has not stained. Lying near and often on the surface of this body is an irregular mass of deep-stained material, comparable possibly to the metachromatic material described above in *Caryoletira*. This has the form sometimes of a compact mass, sometimes of a ring or reticulum. In other parasitized nuclei the spores were more numerous and not so large as in this one figured, measuring about  $5\mu$ .

A parasite similar to the one consisting of aggregates of vesicles in *T. saepicula* was observed, associated with a *Nucleophaga*-like parasite, in *T. quasilla* of *Kalotermes perezii*.

#### DISCUSSION OF THE TAXONOMIC RELATIONSHIPS OF THE NUCLEAR PARASITES OF TRICHONYMPHA

Micrococcaceae have on a number of occasions been reported as parasites in the cytoplasm of protozoa (see Kirby, 1941). In some instances, what were at first described as micrococci were later considered to be *Sphaerita*. The deep-stained homogeneous spores of the chytrid, grouped in sporangia, may appear to be groups of cocci. But some accounts deal with organisms that are clearly not *Sphaerita*; and bacteria of this type are probably much more widespread as parasites of the cytoplasm of protozoa than reports indicate. Only one species has been given a name—*Micrococcus batrochorum* Yakimoff (1930) from *Tritrichomonas batrachorum*. (The species name is given as spelled by Yakimoff. A typographical error in my earlier mention of this, 1941 p. 1032, rendered the accompanying *sic* meaningless.)

MacKinnon (1914), describing a micrococcus parasitic in the cytoplasm of *Entamoeba (Loschia) hartmanni*, reported that it may also invade the nucleus. The parasitized nucleus, she stated, "swells up enormously and the appearance it presents vividly recalls Dangeard's description of the nucleus of *Euglena deses* parasitized by *Caryococcus hypertrophicus*." The enlarged nucleus figured by MacKinnon is partly filled with elongated granules. She stated in a footnote, however, that the nuclear parasite "may not be the micrococcus, but a stage of *Nucleophaga*." Dangeard's observations on *Caryococcus* have been recounted above (page 236).

When the nuclear parasites occur as individual, separately dividing bodies, coccuslike in size and shape, they may reasonably be regarded as cocci and assigned, tentatively at least, to the genus *Caryococcus*.

As was brought out by Lavier (1935), and as discussed by me (1941), some accounts of *Nucleophaga* report individual division of granule-like parasites, with no multinucleate phase. If in truth there is in these forms no multinucleate cytoplasmic mass, the parasites probably are Micrococcaceae rather than *Nucleophaga*.

That genus, like *Sphaerita*, is properly restricted to forms characterized at certain periods of the life history by a multinucleate thallus, and in whose later periods the spores, which may simulate micrococci in many respects, are confined by a sporangial membrane. This membrane is, however, applied to the nuclear membrane in *Nucleophaga*. It is therefore difficult, in the kinds of preparations to which studies of these nuclear parasites have been restricted, to ascertain whether it is present or not.

In none of the organisms previously described as micrococci in protozoa has the structural detail noted in some of the forms in *Trichonympha* been described. Some of those shown by Nägler (1910) in the cytoplasm of *Amoeba* sp. appear to be more deeply stained at the periphery, but this may be an accident of drawing.

*Caryococcus nucleophagus* appears homogeneous in most preparations, but in some, stained with iron-haematoxylin, there appears in optical section a heavy rim extending halfway around the circumference (pl. 13, figs. 23, 27). This varies in thickness, and sometimes there are granules in addition. *Caryococcus invadens* shows also, in some instances, deep-staining and light-staining areas, the former usually extending part-way around the periphery, and often there are one or more central or peripheral granules (pl. 14, fig. 37). A deep-stained area, usually spread in crescentic form along the membrane, but sometimes in the form of a granule at one end or in the central part, is present in *Caryococcus dilatator* (pl. 14, fig. 42; pl. 19, fig. 93). The most notable structure is that of *C. cretus*, with a deep-staining, crescentic peripheral bar of characteristic form (pl. 14, fig. 44). Sometimes there are two of these bars, or a number of granules may replace the bar (pl. 15, figs. 45-46).

In the differentiation of the contents of these small, spherical bodies into deeper-stained and clearer protoplasmic materials, as well as in the intranuclear parasitism, there is a similarity to *Erythrocytonucleophaga ranae* Ivanić, 1934. That granule-like organism, which apparently has a diameter of about  $1-2\mu$ , is reported to invade the red blood cells of *Rana esculenta*, occurring first in the cytoplasm and then in the nucleus. Multiplication is said to take place in the nucleus by binary fission, although the author stated that after the parasite has entered the nucleus its fate could not be followed, because of the heavy staining of the chromatin and plastin substance. There is, indeed, no convincing evidence that this structure, named *Erythrocytonucleophaga*, actually is a nuclear parasite.

The structure of this parasite of the erythrocytes of *Rana* provided Ivanić with a basis for concluding that it is a protozoan. The presence of a large, deep-staining, nucleuslike granule does not necessarily, however, show that it belongs in that group. Students of the cytology of bacteria have reported this type of structure in various Micrococcaceae, as well as in other Schizomycetes. The size, position, and form of the body described by Mencl (1910) as the nucleus of *Micrococcus* resembles very closely the structure Ivanić showed; and a small, round, centrally located granule had been represented (as the nucleus) by Nakanishi (1901) in *Staphylococcus* and other Micrococcaceae. Dobell (1911) described a large, centrally situated body, which he called a nucleus in Micrococcaceae from the intestine of lizards and toads. Pettit (1927) found one or two staining granules in the cells of intestinal cocci and *Sarcina*, but regarded them not as nuclei but as metachromatic corpuscles. Delaporte (1939) studied the structure of fifteen species of Coccaceae, along with many other bacteria, and reviewed all previous work dealing with the cytology of bacteria. The coccus and sarcina forms that she studied included parasitic types from the caecum of the guinea pig. She found that all showed essentially the same structure, and concluded that the structure is general for cocci. There is a central

chromatic body which stains with the customary nuclear stains and is positive to the Feulgen reaction. In the cytoplasm of certain cells are also one or two metachromatic granules, and in all the cells one or many lipid spherules.

The peripheral position occupied by the stained granules or elongated bodies in certain of the forms observed in the nucleus of *Trichonympha* recalls the reports of Mencl (1911), Enderlein (1925), and Stoughton (1929). Mencl stated that in older cultures of *Azotobacter chroococcum* coccus forms were observed containing a variable number of granules, or an elongate flattened body, located at the periphery. Enderlein wrote of the peripheral position, and often the flattening against the membrane, of the "mych," of which *Micrococcus*, he stated, possesses a single one; and Stoughton showed a deep-stained granule applied to, and in some instances flattened against, the periphery in coccuslike forms produced in *Bacterium malvacearum* (*Phytomonas malvacearum*).

A critical consideration of the cytology of bacteria is not in the province of the present work, nor is the material of the nuclear parasites of *Trichonympha* adequate to constitute a significant contribution to this subject. A comprehensive review of the cytology of bacteria has recently been given by Lewis (1941). The facts pointed out above are intended to indicate only that what I have observed in micrococci of the nucleus of *Trichonympha* is not in disagreement with the results of more complete studies of bacteria of this group; and to support the allocation of these parasites to the Micrococcaceae.

The nuclear parasites of *Trichonympha* placed here in the genus *Caryoletira* show certain affinities to the nuclear parasites of Protozoa that have been placed in the genus *Nucleophaga*. The latter genus has been considered to be very close to, if not identical with *Sphaerita*. *Sphaerita* and *Nucleophaga* are included in the family Olpidiaceae of the Chytridiales (Fitzpatrick, 1930; Sparrow, 1943). Members of the family Olpidiaceae are characterized by the possession of flagellated zoöspores, and such zoöspores have been found in many, but not all, of the forms of *Sphaerita* in free-living Protozoa. In *Nucleophaga*, however, and in *Sphaerita* of endozoic Protozoa, zoöspores have not been found to be flagellated. The spores of the *Nucleophaga*-like parasites of *Trichonympha*, and of *Caryoletira*, are not provided with flagella.

In comparing the Haplosporidia and Chytridiales, Caullery and Mesnil (1905) called attention to the fact that the spores of the fungus group are zoöspores with flagella, whereas those of the Haplosporidia lack flagella. This fact, by itself, cannot be used as the criterion for the assignment of the organisms now under consideration to one or the other of these groups. The loss of flagella from zoöspores of certain Chytridiales, in response to special environmental conditions, would not be an unnatural development. But the fact that flagellated zoöspores have not been found, together with the incompleteness of our knowledge of the life histories of most of the nuclear parasites, leaves the genus *Nucleophaga* in uncertain position.

In the size and structural characteristics of the spores there is much difference between typical *Nucleophaga* and *Caryoletira*. (An apparent transition between the two genera is, however, found when we consider the nuclear parasite of *Caduceia theobromae*, which I assigned (1941) to *Nucleophaga*, and the *Nucleophaga*-like parasites of *Trichonympha* described in this paper.)

Typical of *Caryoletira* is the presence of a massive nucleus displaced toward one end, and a greater or lesser abundance of metachromatic substance in the cytoplasm. Structures of this character are found in Acnidosporidia, both in the Sarcosporidia and Haplosporidia.

In agreement with the Haplosporidia, and also the Chytridiales, *Caryoletira* has

a vegetative plasmodial phase with multiplication of nuclei, and at the end of the vegetative phase spores are formed. I cannot say whether there is proliferation in the vegetative phase. It seems likely that there is only sporogony. *Caryoletira* is excluded from the family Haplosporidiidae by various cytological features, and the spore lacks the orifice and valve. There is no affinity to the Metchnikovellidae. The resemblance is closer to the Bertramiidae and Coelosporidiidae, although for the most part in negative characters.

In making comparisons between *Caryoletira* and other forms I am hampered by the limitations of the material, which have precluded a complete understanding of the life history. I can, however, point out certain similarities that I have noted in a survey of the literature. These pertain to the nuclear characteristics and the metachromatic material in the cytoplasm of the spore.

In the massive character of the nuclei in the early multinucleate phases of development there is a resemblance to the condition figured in *Coelosporidium periplanetae* by Ivanić (1926), Georgevitch (1927), and Sprague (1940); in *Ichthyosporidium* by Swarczewsky (1914); in *Caullerya mesnili* by Chatton (1907); and especially in *Bertramia* by Minchin (1903), Warren (1906), and Konsuloff (1912). This massive character might, of course be resolved into more detail by improved technique, both in *Caryoletira* and these other forms, but the similarity in the results of the methods used is noteworthy. The granules found in the extranuclear body of species of *Caryoletira* recall the granules found among the nuclei of *Bertramia* by Minchin and Warren. But the granules in the nuclear parasite of *Trichonympha* are not so numerous.

The usually large size and eccentric position of the nucleus in the spores of *Caryoletira* is in agreement with the situation in *Bertramia asperospora* (Konsuloff, 1912), in *Sarcocystis* (Alexeieff, 1913; Jírovec, 1927; Reichenow, 1928; Breindl and Komárek, 1928), and in *Haplosporidium* (Debaisieux, 1920b; King, 1926; Jírovec, 1936). In some of these forms, too, the nucleus is similar in structural characteristics to that in *Caryoletira*. The mere fact that the nucleus occupies an eccentric position, or even that it is generally similar in structure, does not, of course prove any affinity. But the facts are of value in connection with the relationship indicated by all points taken together.

The accumulation of stainable material in the cytoplasm of the spores or other phases of development of Acnidosporidia has been noted repeatedly. Perhaps most attention has been given to this in *Sarcocystis*, in the spore of which strongly siderophile metachromatic granules are abundant, aggregated in the middle or anterior parts of the cell, where they may fill most of the space (Alexeieff, 1913; Erdmann, 1910, 1914; Jírovec, 1927; Breindl and Komárek, 1928). The last named authors commented on the tendency of the granules to "flow together" as a consequence of manipulation or degeneration—a phenomena noted in *Caryoletira*. In *Bertramia asperospora*, Konsuloff (1912) described the extrusion of chromidia from nuclei in the development of spores, and noted the presence in certain stages of a mass of "chromatin" in the protoplasm. Minchin (1903) showed granules in the cytoplasm of the spores of *Bertramia asperospora*.

It is possible that a similar occurrence of metachromatic substance was noted, but misinterpreted, by Warren (1906) in *Bertramia kirkmani*. He stated that in the formation of the spore, the chromatin of the nucleus "separates from the nucleolar element either in the form of a band or hoop," leaving behind a vesicle or vacuole at one end of the elongated spore. The band or hoop breaks up into "six, eight, or ten masses of chromatin," which are distributed in the cytoplasm of the spore. Warren considered these to be nuclei, the spore thus having 6-10 nuclei,

the greater number of which, he thought, are "absorbed or fuse together" when the sporozoite is transformed into the trophozoite. It is possible, however, that the vacuole may really represent the nucleus, and that the granules in the cytoplasm are metachromatic material. Warren found that the vesicle or vacuole "does not stain at all readily; iodine has no particular effect on it, neither has it any marked affinity for aniline stains or haematoxylin." In *Caryoletira* I have noted that the nucleus may stain less with iron-haematoxylin than the metachromatic granules, and may even remain altogether unstained while those granules are deeply stained.

Aggregates of chromatic granules appear in the cytoplasm in the formation of spores of *Halposporidium* (Granata, 1914; Debaisieux, 1920b; Jirovec, 1936). King (1926) described masses of granules in the sporoblasts and spores of *Haplosporidium chitonis*; he considered them Golgi bodies.

Metachromatic material is abundant in the cytoplasm of the flagellate zoospore of *Coelomycidium simulii*, which seems clearly to belong to the Chytridiales (Debaisieux, 1920a). In the formation of the zoospores, chromatic bodies of varied form appear in the cytoplasm, often forming a semicircle at the periphery. According to Debaisieux, the chromatic bodies condense into two or three large masses and then into a single mass. This structure occurs in the mature zoospores as a relatively large more or less dense body, which stains more deeply with iron-haematoxylin than the true nucleus. Cytoplasmic spherules that stain with iron-haematoxylin are abundant in the spore of *Rhinosporidium scaberi* (Ashworth, 1923).

In free-living nematodes Micoletzky (1925) found a variety of parasites, some of which, so far as one can judge from the much simplified illustrations, resemble *Caryoletira* in certain particulars. In the amoebosporidia described from *Achromadora ruricola*, parasitic in the cells of the intestine, there are cysts containing spherical structures (Micoletzky, pl. 11, fig. 44b) that much resemble the spores of *Caryoletira*. There is a peripheral granule, which Micoletzky referred to as nucleuslike, a dark, apparently granular zone crescentic in optical section, and a hyaline part between this and the nucleus. Micoletzky did not know whether this represented a vegetative stage or sporulation. In the body cavity of *Dorylaimus carteri* he noted spores of 3–5  $\mu$  with contents differentiated into two parts, and in the figures these bear a certain similarity to *Caryoletira* spores. Ovoid spores (about 6–7  $\times$  5  $\mu$ ) with a peripherally located, nucleuslike body occurred in the body cavity of *Dorylaimus carteri*, and in *Theristus dubius* an ovoid cyst packed with spherical bodies (Micoletzky, pl. 13, fig. 59b) recalls a sporangium group in *Caryoletira* and *Bertramia*. In the subcuticular tissue of the same nematode, he noted cysts containing densely packed granules of small size (0.4–0.5 in), possibly small spores. These are suggestive of the clusters of cocci that have been reported from both the cytoplasm and nucleus of Protozoa.

It is perhaps not unlikely that when the nuclear parasites described as *Nucleophaga* are better known, some of them will be found to be cocci, and others will show relationships to these haplosporidian forms of simple structure and life history (Bertramiidae, etc.). The group of Haplosporidia has long been regarded by protozoologists as of uncertain status, but it has generally been retained to receive a group of genera that have not yet been shown to belong elsewhere. With more information, the suggestion of Debaisieux (1920b) and others may be finally adopted; and the order Haplosporidia, having rendered service as a receptacle for provisional allocation of incompletely understood protista, may be eliminated. Until then, it seems best to place *Caryoletira* in the Bertramiidae; and it is necessary to leave unresolved the dislocation thus introduced between this and other chytridlike nuclear parasites of protozoa.



A particular interest attaches to parasitism in the nucleus of cells. The parasite may show a marked organelle specificity, passing through the cytoplasm of the cell and invading the nucleus; and in ciliates there may be a further selection between the macronucleus and micronucleus. Certain parasites may develop in either the nucleus or the cytoplasm, and thus not have developed this specificity, but the majority are exclusively intranuclear for the entire developmental period within the cell. *Eimeria salamandrae* (Steinhaus) is said to develop indifferently in either the nucleus or the cytoplasm (Simond, 1896; Sargent, 1902), but on the other hand, there are several coccidia in which the development of schizonts and gametocytes takes place entirely within the nucleus. In vertebrate hosts, developing in nuclei of the intestinal epithelium, are *Eimeria ranarum* (Labbé) in *Rana esculenta* (Laveran and Mesnil, 1902), *Isospora mesnili* Sargent in *Chamaeleo vulgaris* (Sargent, 1902), and *Cyclospora caryolytica* Schaudinn in moles (Schaudinn, 1902). More recently, an instance of exclusive intranuclear parasitism by a coccidian has been reported in an invertebrate, the polychaete worm *Polydora flava* (Fowell, 1936). *Caryoletira*, the cocci, and the Chytridiales occurring in the nucleus of Protozoa apparently develop only in that organelle. Although certain forms of *Nucleophaga* and *Sphaerita* are so much alike that it would seem to be impossible to state that the same chytrid may not occur both in the nucleus and in the cytoplasm, in practice, so far as my experience has gone, there is generally little confusion. Either a population of a species shows parasites only in the nucleus or in the cytoplasm, or, if the parasites do occur in both places in the same population, differentiation between them is possible.

#### SUMMARY

In the host distribution of many of the species of *Trichonympha* of termites a significant pattern is evident. In it is manifested a considerable degree of resistance to minor structural change, and there is a close relative host specificity. The differences that exist between species are, in general, though not always, of relatively abrupt order. In all five species of *Anacanthotermes* examined, three of them alcoholic specimens, there is a *Trichonympha* of the *turkestanica* type; probably all are this species. *Trichonympha chattoni* occurs in fourteen species of *Glyptotermes*. Flagellates of the genus have been found in only two other species of *Glyptotermes*; each of these contains a one-host species that differs only in minor particulars from *T. chattoni*. *Trichonympha pyclophora* occurs in a number of related species of *Necotermes* from Madagascar and Mauritius. *Trichonympha agilis*, or a closely similar form, is the only one usually found in *Reticulitermes*, where it is present in all the species that have been examined.

A detailed description is given of the morphology of *Trichonympha turkestanica*. In the anterior part of the rostral tube there is a conspicuous central rod, which is not prolonged directly into either of the two crescentic bodies at the anterior end of the tube. A similar, but often less conspicuous, central rod has been observed in *T. ampla*, *T. collaris*, and certain species in *Cryptocercus*; but it appears not to be present in some species of *Trichonympha*. A structure in *T. turkestanica* that has not been observed in any other species of the genus is a ring in the outermost endoplasm a short distance posterior to the mantle of basal granules. The ectoplasm of *T. turkestanica* contains symbionts of three sorts: two types of peripheral granules are always present between the flagellar plates, and some specimens contain a few spindle-shaped organisms in the ectoplasm.

An account is given of the twelve species of *Trichonympha* that have thus far been found in termites of the family Kalotermitidae. Five of these are new. With

the type hosts, they are: *T. divexa* of *Kaloterms* (s.l. new genus?) sp., South Africa; *T. ampla* of *Kaloterms occidentis*, Mexico; *T. corbula* of *Procryptotermes* sp., Madagascar; *T. teres* of *Neotermes meruensis*, Tanganyika; and *T. peplophora* of *Neotermes howa*, Madagascar and Mauritius.

The parabasal filaments and parabasal bodies or cords are described in various species of *Trichonympha* of termites and *Cryptocercus punctulatus*. The filaments from the parabasal cords to the region of the circular fissure, at the base of the rostral tube, are usually situated peripherally in the endoplasm for their full length; and their connection with the parabasal bodies is at one transverse level. Parabasal bodies originate peripherally at various transverse levels in *T. turkestanica* and *T. peplophora*. In some species the anterior ends of the parabasals are aggregated in the central region of the endoplasm, and the parabasal filaments are gathered in a central group. In many species part or all of the parabasal cords constitute a suspensory apparatus for the nucleus. In *T. grandis* a central group of filaments of another sort is present; these pass around the nuclear membrane and continue free in the cytoplasm posterior to the nucleus. The group corresponds to the nuclear sleeve previously described; they appear to be equivalent to the axostylar filaments of certain hypermastigotes. Similar filaments have been observed in *T. algoa* and *T. quasilla*.

There are distinct differences, often of relatively large order, in the arrangement of the parabasal bodies among species or groups of species; and the form of the parabasal apparatus is essentially constant within a species. It is the most valuable characteristic for use in analyzing speciation in this genus.

Observations have been made on certain extranuclear structures in division of eight species of *Trichonympha*. After separation of the halves of the rostral tubes, a new rostral tube half develops as a narrow lamella in relation to the anterior end of each. From early in development of this, short new flagella are present on its outer surface. The definitive structure of the new rostrum is not attained until after division of the cytosome.

In species of *Trichonympha* the position of the poles of the division figure is not uniform. In one group of species the spindle poles are associated with the posterior ends of the old rostral tube halves. This group includes *T. turkestanica*. The central rod in the rostral tube of that species is present and associated with one tube half throughout division; a new one develops early in the other tube half. This rod retains its original size and has no relation to the spindle; thus, although it occupies the same position as the rod designated as a centriole in species of *Trichonympha* in *Cryptocercus*, it cannot be regarded as a centriole.

In another group of species each pole of the spindle is associated with the new rostral tube half, and is nearer the anterior than the posterior end of the old half. The relation is to a bend in the anterior part of the new structure, without direct contact. In *T. ampla* a short rod, separate for most of its length from the new tube half, extends from the spindle pole to the anterior granule from which the tube half arises. A corresponding structure may parallel the anterior part of the new tube half in *T. peplophora*; nothing equivalent has been seen in certain other species. The reported observations on the achromatic structures in division of *Trichonympha* of termites are inconclusive, and await further analysis for correlation with the situation in other hypermastigotes, particularly as regards the centriole relationship.

In division of *T. chattoni* the flagellar plates are distributed into two approximately equal groups; new plates to restore the original complement develop after plasmotomy. The parabasal bodies seem to be distributed intact in two groups in *T. chattoni*. In *T. turkestanica*, however, the substance of the parabasal bodies dis-

integrates early in the prophase, leaving only the filaments. New parabasal bodies appear after division of the cytosome.

A survey has been made of various kinds of parasites in the nucleus of *Trichonympha* of termites. Micrococcaceae are considered under four new species: *Caryococcus nucleophagus* of *Trichonympha corbula*, *Caryococcus invadens* of *Trichonympha peplophora*, *Caryococcus dilatator* of *Trichonympha chattoni* and many other species, and *Caryococcus cretus* of *Trichonympha corbula*. *Nucleophaga*-like parasites are described from several species. The new genus *Caryoletira* is established with the type *C. anulata* of *Trichonympha corbula*. A relationship is suggested between this organism and Haplosporidia of the family Bertramiidae. The spores of *Caryoletira* are relatively large in size, being very much larger than those of *Nucleophaga*, and they are provided with eccentric nuclei and more or less prominent metachromatic granules. A second species, *Caryoletira magna*, is established for a parasite of *Trichonympha turkestanica* with still larger spores.

The nucleus becomes greatly enlarged, and the chromatin disappears, in instances of parasitization by *Caryococcus dilatator*, the *Nucleophaga*-like forms, and *Caryoletira*. The other species of *Caryococcus* do not cause such enlargement of the invaded nuclei or disappearance of chromatin.

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## PLATES

Abbreviations for methods of preparation: B, Bouin's fluid; D., Delafield's haematoxylin; Er., erythrosin; F., acid fuchsin; Fl., Flemming's fluid; H., Heidenhain's haematoxylin; Holl., Hollande's fluid; PS., protein silver (protargol); R., Regaud's haematoxylin; S., Schaudinn's fluid; Su., Susa fixative of Heidenhain.



## PLATE 12

Figs. 1-5. Non-parasitized nuclei of *Trichonympha corbula* from *Procryptotermes* sp., Madagascar. 4, S.D.  $\times 2600$ ; others, S.H.F.,  $\times 1750$ .

Fig. 1. Chromatin mass somewhat contracted; small nuclear granules and radial strands peripheral to this; nucleolus, with deeper-staining shell.

Fig. 2. Chromatin in irregularly branched, peripheral strands; nucleolus interior to the peripheral chromatin. Surface view.

Fig. 3. Surface view of nucleus similar to that represented by figure 2. Stouter peripheral strands of chromatin; interior nucleolus and nuclear granules.

Fig. 4. Nucleus of a type frequently observed in Schaudinn-fixed material; reticular chromatin mass somewhat contracted from membrane; nuclear granules at periphery and space between these and chromatin; nucleolus peripheral to chromatin mass.

Fig. 5. Optical section. Chromatin mostly peripheral, with some strands of chromatin extending into the interior; nucleolus interior; inner part of nucleus occupied by nuclear granules and fibrils.

Figs. 6-16. Non-parasitized nuclei of *Trichonympha chattoni* from *Glyptotermes montanus*, showing variations in the nucleolus. 13,  $\times 1800$ ; others,  $\times 1750$ .

Fig. 6. Chromatic granular mass, no nucleolus observed. S.D.

Fig. 7. Chromatic mass in strands, which are paired in places. Nucleolus appears as a solid body. C.R.

Fig. 8. Nucleolus of normal size, vesicular, with a large central granule. S.D.

Fig. 9. Nucleolus larger, vesicular, with a large granule. C.R.

Fig. 10. Nucleolus much enlarged, vesicular, containing a large vacuolated mass. S.P.

Fig. 11. Nucleolus small and solid in appearance. S.D.

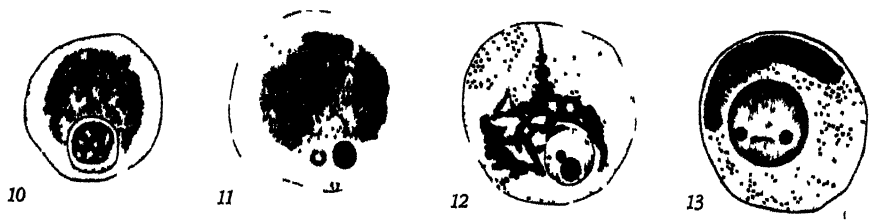
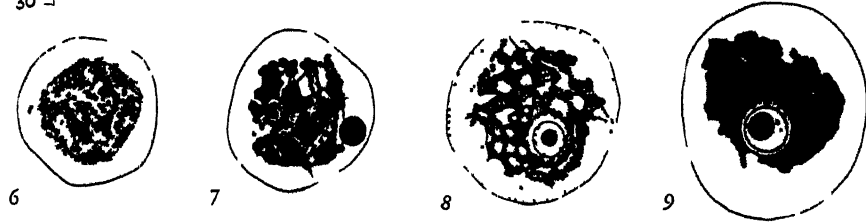
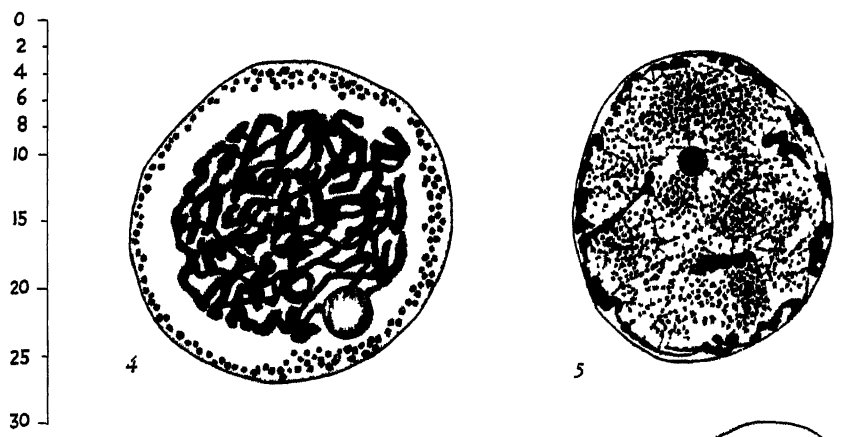
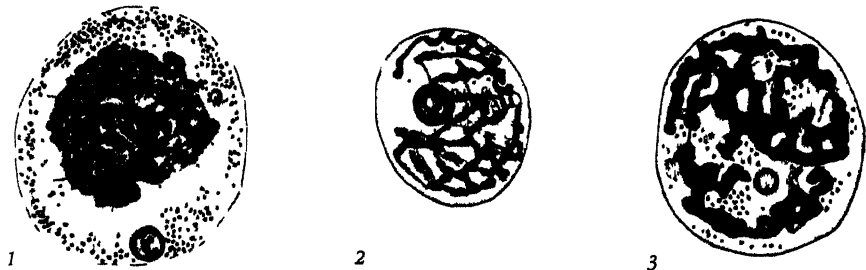
Fig. 12. Chromatin contracted in a reticular mass; nucleolus large, with one large and one small interior granule. S.D.

Fig. 13. Chromatin in optical section, contracted on one side in a crescentic area; nucleolus very much enlarged, vesicular, with two granules. S.D.

Fig. 14. Chromatin massed at one edge of nucleus; nucleolus much enlarged, containing a chromatic strand. Like most of the preceding nuclei, the area not occupied by the chromatin or nucleolus contains small nuclear granules. S.D.

Figs. 15-16. Late prophase nuclei; chromosomes in pairs. The vesicular nucleolus, containing several granules, is still present. S.D.

Scale  $\times 1750$ .



## PLATE 13

Nuclei of *Trichonympha corbula* from *Procrystotermes* sp., Madagascar, parasitized by *Caryococcus nucleophilus*.

Fig. 17. Dense chromatin mass and radial fibrils. Only a few parasites present, peripheral to chromatin mass. Holl. II.  $\times 2600$ .

Fig. 18. Parasites in pairs, all peripheral to chromatin mass. Holl. II.  $\times 2600$ .

Fig. 19. Chromatin mass excessively contracted, with dense spherical nucleolus peripheral to it. Numerous parasites present in peripheral region. F.R.  $\times 2700$ .

Figs. 20–21. Parasites, some of which are in pairs, present both exterior to and within the mass of chromatin. F.R.  $\times 2600$ .

Fig. 22. Parasites mostly interior to the reticular chromatin mass. S.D.  $\times 2600$ .

Fig. 23. Chromatin restricted to the periphery, parasites occupying central region. Some of the parasites show a peripheral, crescentic area deeper-staining than the rest. F.R.  $\times 2700$ .

Fig. 24. Similar to preceding; nucleolus appears as a ring; parasites are mostly in pairs or show phases of fission. F.R.  $\times 2700$ .

Fig. 25. Detail from figure 24, showing successive phases of fission. F. R.  $\times 5400$ .

Fig. 26. Chromatin peripheral, parasites in a compact inner group. S.D.  $\times 2600$ .

Fig. 27. Parasites; some paired, showing peripheral, deep-staining area on one side. F.R.  $\times 2600$ .

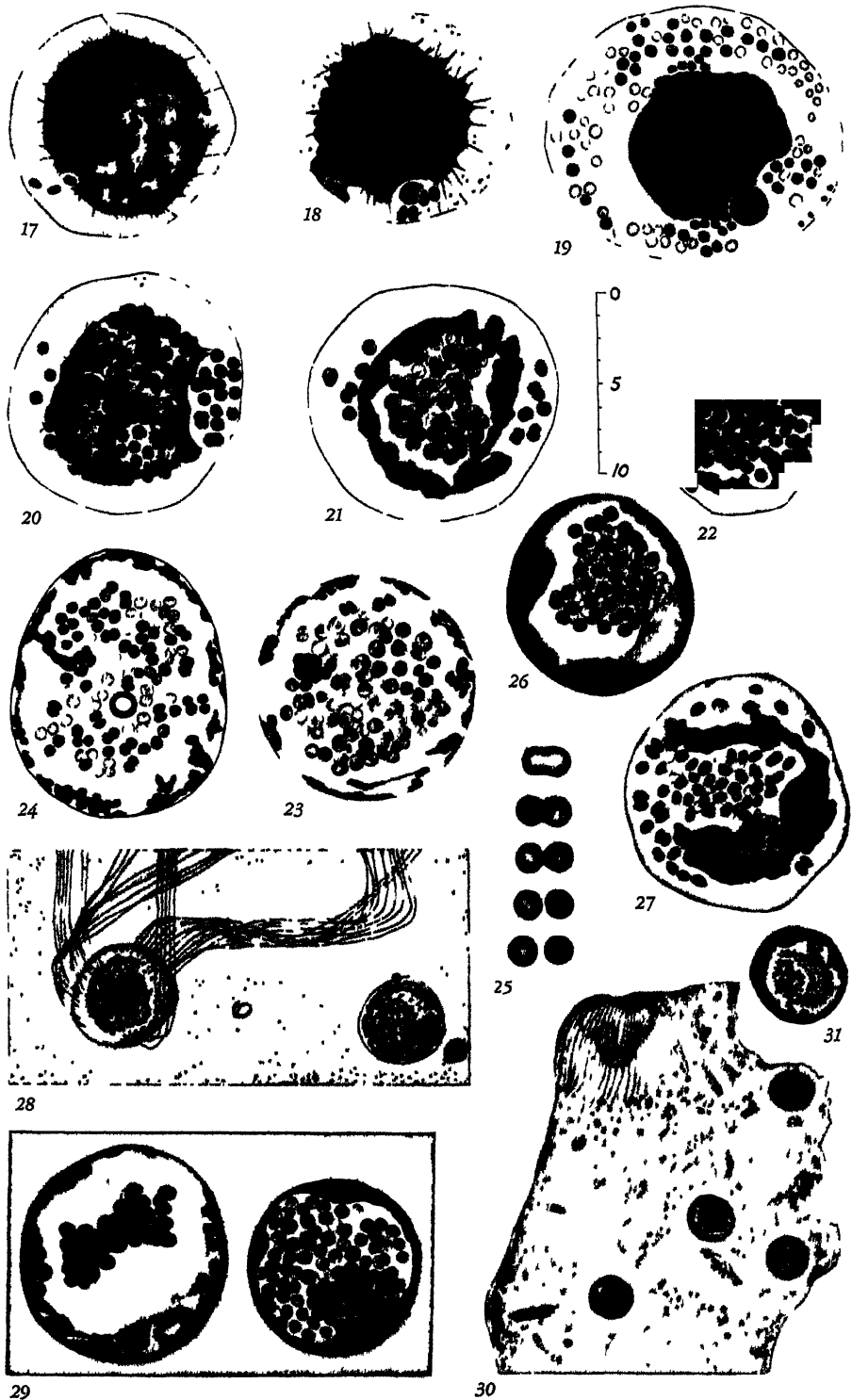
Fig. 28. Two nuclei in one *Trichonympha*. The left one is in the usual position, in association with the parabasal bodies. Parasites are present peripheral to the chromatin mass in that nucleus, interior to the chromatin in the other. S.D.  $\times$  app. 1275.

Fig. 29. Two nuclei in one *Trichonympha*, both with peripheral chromatin and an interior group of parasites. S.D.  $\times 2600$ .

Fig. 30. Four nuclei in one *Trichonympha*, all parasitized. S.D.  $\times 480$ .

Fig. 31. Enlargement of one nucleus from figure 30.  $\times 1275$ .

Scale  $\times 2600$ .



## PLATE 14

Fig. 32. *Trichonympha corbula* from *Procryptotermes* sp., Madagascar, with six nuclei, all parasitized by *Caryococcus nucleophagus* interior to the peripherally located chromatin. S.D.  $\times 845$ . At the right are enlarged figures of the nuclei.  $\times$  app. 1275.

Figs. 33-35. *Caryococcus nucleophagus* in nucleus of *Trichonympha corbula* from *Kaloterme* (s.l.) *castaneiceps*.

Fig. 33. Parasites peripheral to the contracted chromatin mass, each showing deep-stained area at one side. S.H.  $\times 1800$ .

Fig. 34. Parasites peripheral to dense chromatin mass. S.D.  $\times 1750$ .

Fig. 35. Parasites peripheral to the contracted chromatin and also within the nucleolus, which is hypertrophied. S.H.  $\times 1800$ .

Figs. 36-39. *Caryococcus invadens* in nuclei of *Trichonympha ptyophora* from *Neotermes howa*.

Fig. 36. Parasites around the chromatin mass are in pairs. Parasites are present within the enlarged nucleolus. Holl. H.  $\times 1750$ .

Fig. 37. Parasites around chromatin mass showing structural detail, in deep-staining regions and granules. The nucleolus is parasitized. S.H.  $\times 1750$ .

Fig. 38. Enlarged figure of the parasitized nucleolus of figure 38. Shows a chromatic shell and granules in addition to the parasites.  $\times 3500$ .

Fig. 39. Parasites in division stages and in pairs. The spherical group of parasites are those that have developed in nucleolus. S.D.  $\times 1750$ .

Figs. 40-42. *Caryococcus dilatator* in nucleus of *Trichonympha ptyophora* from *Neotermes howa*.

Fig. 40. The chromosomes have entered upon a normal prophase organization, but the nucleolus is parasitized and enlarged. S.D.  $\times 1750$ .

Fig. 41. Parasites are present in the peripheral region and in the enlarged nucleolus. Holl. H.  $\times 1750$ .

Fig. 42. The nucleus is greatly enlarged, and is entirely occupied by parasites except for the remnant of chromatin in the center. S.H.  $\times 1750$ .

Fig. 43. *Caryococcus dilatator* in nucleus of *Trichonympha chattoni* from *Glyptotermes iridipennis*. Nucleus enlarged and filled with parasites except for some strands of chromatin, which extend among the bacteria. Optical section. S.D.  $\times 1750$ .

Fig. 44. *Caryococcus cretus* in nucleus of *Trichonympha corbula* from *Procryptotermes* sp., Madagascar. S.D.  $\times 2600$ .

Scale  $\times 1750$ .

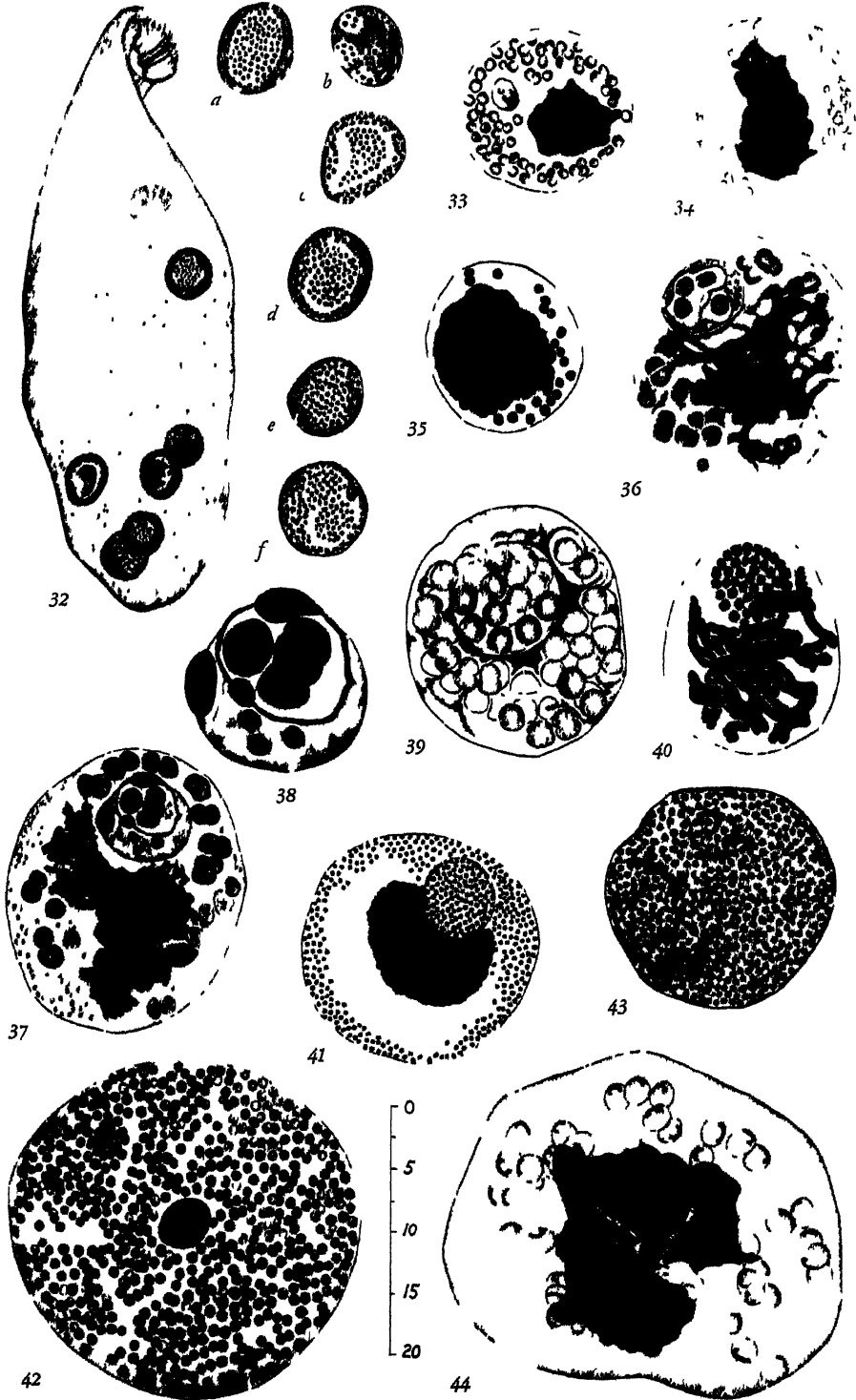


PLATE 15

Figs. 45-46. *Caryococcus erlus* in nucleus of *Trichonympha corbula* from *Pro-cryptotermes* sp., Madagascari.

Fig. 45. Parasites in the spaces of the reticulum of chromatin; each contains a sharply defined, curved, deep-staining body. S.II.  $\times$  2600.

Fig. 46. Optical section. Peripherally located chromatin strands, with some strands extending into interior; nucleolus with more deeply staining outer shell; parasites occupying interior. S.II.  $\times$  2700.

Figs. 47-57. *Nucleophaga*-like parasites in nucleus of *Trichonympha chattoni* from *Glyptotermes montanus*.

Fig. 47. Two vacuolated, irregular bodies in nucleus; chromatin disarranged but abundant. S.D.  $\times$  1800.

Fig. 48. A number of bodies, variable in size; chromatin reduced in amount and arranged in a belt of granules. S.D.  $\times$  1800.

Fig. 49. Parasites in earlier phase, as in above two figures, vacuolated bodies filling nucleus, which is not enlarged. S.D.  $\times$  1800.

Fig. 50. Chromatin peripheral, parasite interior. S.D.  $\times$  1800.

Fig. 51. Immature parasites. Optical section, showing homogeneous spherules among which are granules and strands of chromatin. S.II.  $\times$  1750.

Fig. 52. Similar phase, surface view; group of spherules with chromatin strands among them. S.H.  $\times$  1750.

Fig. 53. Mature spores, varying in size, each with large, deep-stained, nucleus-like body. S.H.  $\times$  1750.

Fig. 54. Detail of one of the above spores, nucleus rounded, at one side, showing indications of granular structure. Granules in cytoplasm. S. II.  $\times$  2600.

Fig. 55. Enlarged nucleus with mature spores, each with a peripheral, deep-staining granule. Some vestiges of chromatin. S.H.  $\times$  1800.

Fig. 56. Ruptured nucleus, from which spores are escaping. The body of the flagellate in which this occurred had also been ruptured, but specimens near by were in good condition. C.R.  $\times$  1750.

Fig. 57. *Trichonympha chattoni* from *Glyptotermes montanus*, with nucleus in position but ruptured, homogeneous spherules dispersed in cytoplasm. S. II.  $\times$  530.

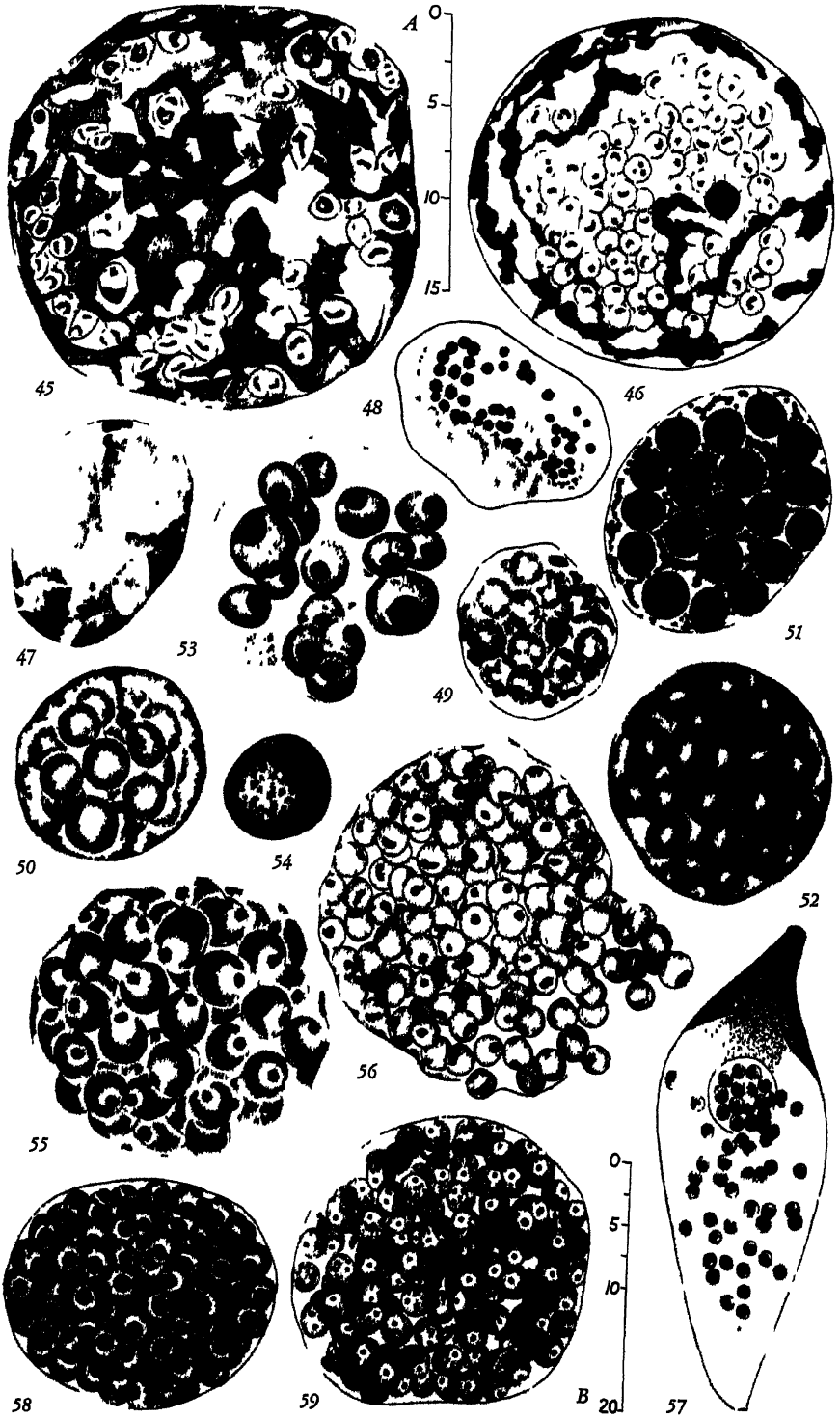
Figs. 58-59. *Nucleophaga*-like parasites in nucleus of *Trichonympha chattoni* from *Glyptotermes iridipennis*. S.II.  $\times$  1750.

Fig. 58. The nucleuslike granule is unusually large; no chromatin is left. S. II.  $\times$  1750.

Fig. 59. The nucleuslike granule is very small. Strands of chromatin extend among the spores. S.II.  $\times$  1750.

Scale A,  $\times$  2600.

Scale B,  $\times$  1750.





## PLATE 16

Figs. 60-75. *Caryolitia anulata* in nucleus of *Trichonympha corbula* from *Pro-cryptotermes* sp., Madagascar.

Fig. 60. Optical section, earlier phase of parasitization; numerous homogeneous spherules; strands of chromatin are present at the periphery, and some extend among the spherules; chromatic granules among the spherules. Holl. H.  $\times 2700$ .

Fig. 61. Optical section of similar stage; the chromatin is all in a peripheral reticulum; the homogeneous spherules are grouped in the interior of the nucleus. S.H.  $\times 2600$ .

Fig. 62. Surface view; the parasites appear as homogeneous, ellipsoidal bodies interior to the reticulum of chromatin; some of the bodies show evidence of constriction. Holl. H.  $\times 2600$ .

Figs. 63-64. Surface view and optical section of the same nucleus; peripheral reticulum of chromatin, homogeneous bodies occupying interior, chromatic granules among these bodies. Holl. H.  $\times 2600$ .

Fig. 65. Nucleus containing an unusually small number of spores (17). The nucleus is in the normal position in *Trichonympha* and is only  $13\mu$  in diameter. Holl.  $\times 2600$ .

Fig. 66. Spore of the group represented in figure 65. At the upper end is a beehive-shaped body, composed of granules, probably the nucleus. Near the middle is a ring of cytoplasmic granules.  $\times 5200$ .

Fig. 67. Spore in which, because of poor fixation, the chromatic masses appear solid. Anteriorly is a nuclear body, in the middle is a solid equatorial ring, posteriorly is a mass of other cytoplasmic material. Fl. R.F.  $\times 5400$ .

Figs. 68, 69. Spores from one parasitized nucleus, similar in nuclear bodies and equatorial rings, different in size and distribution of posterior cytoplasmic granules. Holl. H.  $\times 5200$ .

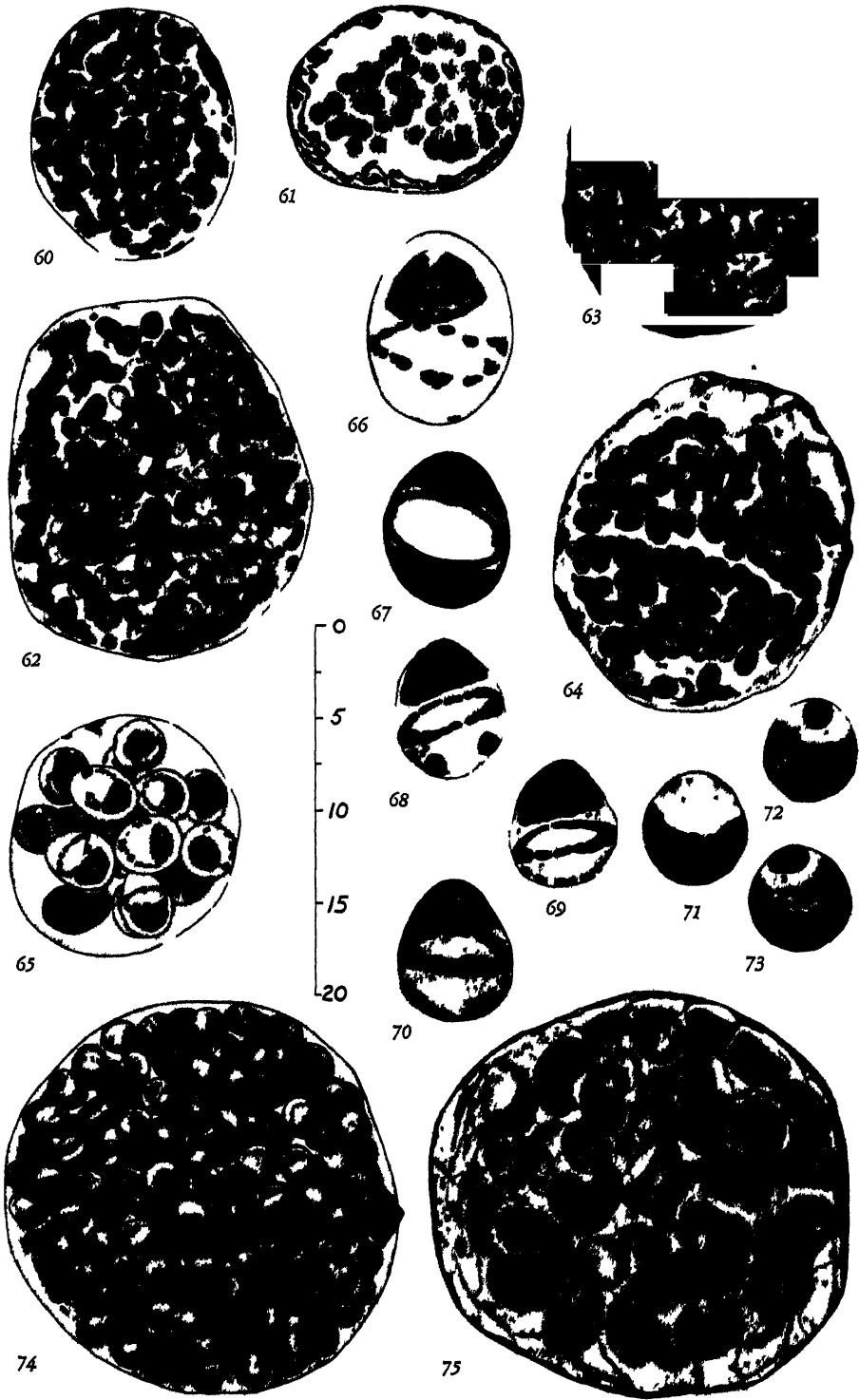
Fig. 70. Spore with nuclear body applied to the membrane, unlike figure 66, where it is separated by a space; equatorial ring of granules, and area of granules at periphery perpendicular to this. Holl. H.  $\times 5400$ .

Fig. 71. Spore from a nucleus greatly enlarged as in figure 74, and similarly containing a very large number of spores and a crystalloid body. The spores showed only a clear area at the end, where others showed the nuclear body; in the other half is a large group of granules. Holl. H.  $\times 5400$ .

Figs. 72, 73. Spores from a similarly enlarged nucleus, with a crystalloid body, on the same slide as figure 71. In each spore there is a small, compact nuclear body. Holl. H.  $\times 5400$ .

Fig. 74. Greatly enlarged, displaced nucleus crowded with spores and containing a peripheral, hexagonal, bipyramidal crystalloid body. This produces a protuberance of the membrane at one point. Holl. H.  $\times 1750$ .

Fig. 75. Parasitized nucleus with spores poorly fixed and stained, but possibly not yet mature. Strands of chromatin are present at the periphery of the nucleus, whereas in nuclei with mature spores there are few or no vestiges of chromatin. S.D.  $\times 2600$ .



## PLATE 17

Figs. 76-82. *Caryolctua anulata* in *Trichonympha corbula* from *Procyptotermes* sp., Madagascar.

Fig. 76. Entire *Trichonympha*, showing great enlargement and displacement of nucleus containing mature spores. Holl. H.  $\times 860$ .

Fig. 77. Nucleus ruptured, spores dispersed in cytoplasm. Holl. H.  $\times 1000$ .

Fig. 78. A small part of the parasitized nucleus attached to the parabasal bodies and nearly separated from the rest; crystalloid body in posterior part of nucleus, producing protuberance of membrane. Holl. H.  $\times 860$ .

Fig. 79. Nucleus containing mature spores and peripheral crystalloid body, which is seen in cross section. Above is an enlargement of the crystalloid, showing its hexagonal shape. Holl. H.  $\times 860$  and  $2600$ .

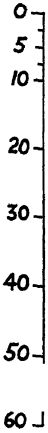
Fig. 80. Parasitized nucleus containing homogeneous bodies, vestiges of chromatin and a small diamond-shaped body which is probably an early developmental phase of the crystalloid. Holl. H.  $\times 860$ .

Figs. 81-82. Nuclei with mature spores, each showing in side view the diamond-shaped crystalloid; in cross section the crystalloids are hexagonal as in figure 79. Holl. H.  $\times 860$ .

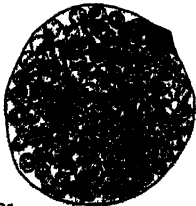
Scale  $\times 860$ .



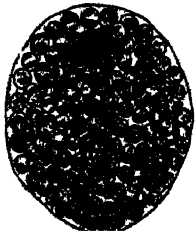
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81



82

## PLATE 18

Figs. 83-91. Parasites similar to *Caryoletta anulata*.

Fig. 83. Parasitized nucleus of *Trichonympha corbula* from *Kalotermes* (s.l.) *longus*. Strands of chromatin are present at the periphery. F.R.  $\times 2600$ .

Fig. 84. Parasitized nucleus of *Trichonympha corbula* from *Kalotermes* (s.l.) *castaneiceps*. No crystalloid body, as shown in figure 74, is present. S.II. F.  $\times 2600$ .

Figs. 85-91. From *Trichonympha peplophora* of *Necotermes howa*.

Fig. 85. Parasitized nucleus attached to parabasal cords, with membrane drawn out in places. S.H.  $\times 860$ .

Fig. 86. Very much enlarged nucleus,  $59\mu$  long, distorted and crowded by mature spores varying in diameter from  $1.7$  to  $5.2\mu$ . S.II.  $\times 860$ .

Fig. 87. Protuberances of membrane over spores, probably not a consequence of fixation shrinkage. S.D.  $\times 1750$ .

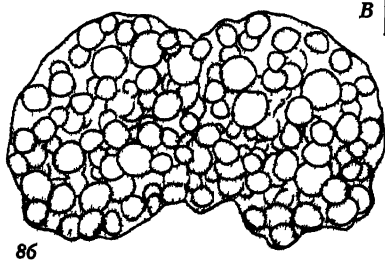
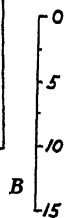
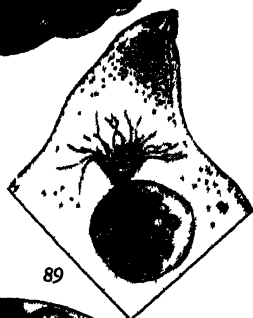
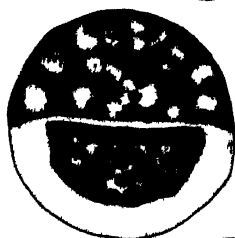
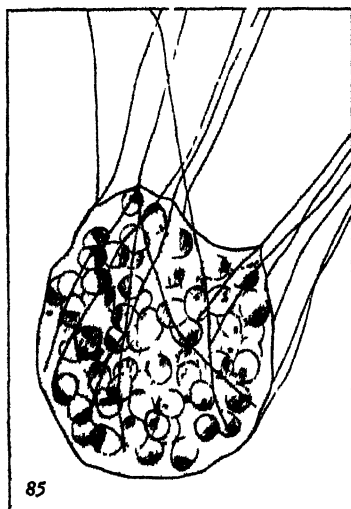
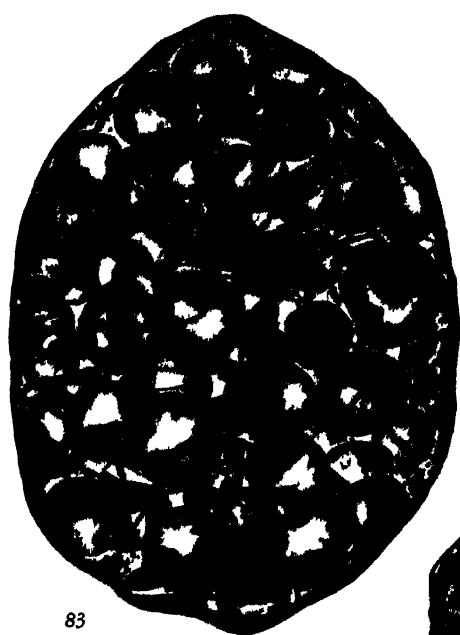
Fig. 88. Detail of spores, from a much enlarged nucleus with many. The nuclear body is a group of well-defined granules. The other half of the spore consists of alveoli and granules. S.H.  $\times 2600$ .

Fig. 89. The nucleus is displaced posteriorly, but a small part, containing one spore, is still associated with the parabasal bodies and is in contact with the rest by a narrow bridge. Holl. H.  $\times 450$ .

Fig. 90. Spores small in number and unusually large in size, the largest  $8.5$  by  $8\mu$ . Holl. H.  $\times 1750$ .

Fig. 91. Semidiagrammatic enlargement of spore from group in figure 90. The hemispherical nuclear body is below, and contains well-defined granules. The upper half is occupied by a vacuolated and granular mass of cytoplasmic materials. Holl. H.  $\times 2600$ .

Scale A,  $\times 2600$ ; B,  $\times 1750$ .



## PLATE 19

Fig. 92. *Caryococcus dilatator* in nucleus of *Trichonympha saepicula* from *Rugitermes kirbyi*. Parasites are present in the much hypertrophied nucleolus, as well as among the chromatin strands. S.H.F.  $\times 1750$ .

Fig. 93. *Caryococcus dilatator* in nucleus of *Trichonympha turkestanica* from *Anacanthotermes ochraceus*. Nucleus hypertrophied and filled with the parasites. Remnants of chromatin in strands. S.H.  $\times 1750$ .

Figs. 94-95. Parasite in nucleus of *T. saepicula* from *Rugitermes kirbyi*. S.H.  $\times 1750$ .

Figs. 96-101. *Caryoletira magna* in nucleus of *Trichonympha turkestanica* from *Anacanthotermes ochraceus*.

Fig. 96. A few spherules among the strands of chromatin. Structural detail within the enlarged nucleolus. Compare plate 12, figs. 8-16. S.H.F.  $\times 1275$ .

Fig. 97. More numerous spherules, some possibly in division. S.H.  $\times 1750$ .

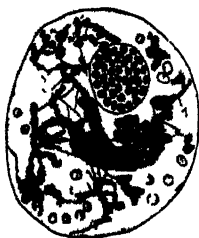
Fig. 98. Many spherules, in nucleus from which the chromatin mass is being partly extruded—an abnormal occurrence. S.D.  $\times 1750$ .

Fig. 99. Spores in development. The deep-stained strands or rows of granules, in some instances segregated into two groups, suggest mitotic division. S.H.F.  $\times 1275$ .

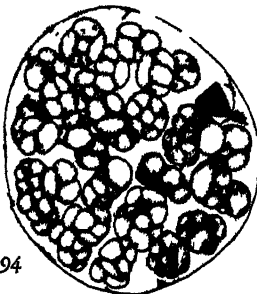
Fig. 100. Mature spores, more deeply stained nucleus and vacuolated mass of metachromatic substance. S.H.  $\times 860$ .

Fig. 101. Single spore, from a group similar to that of figure 100. Metachromatic mass, occupying most of the interior, more deeply stained than the nucleus. S.H.  $\times 1750$ .

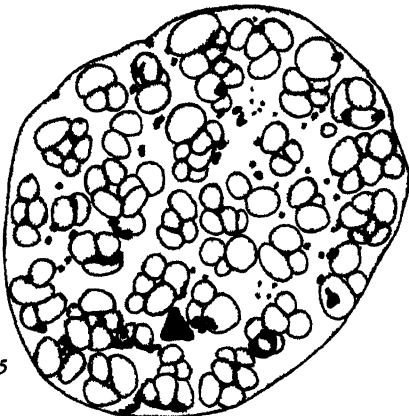
Fig. 102. *Caryoletira*-like spores in a nucleus of *Trichonympha saepicula* from *Rugitermes kirbyi*. S.H.F.  $\times 1750$ .



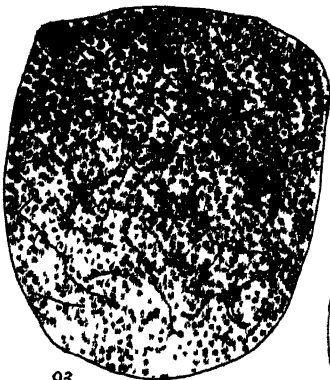
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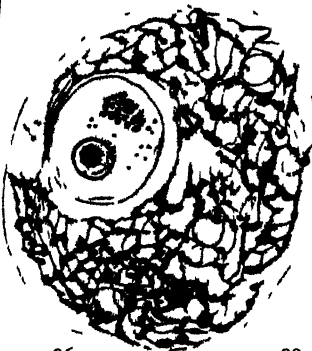
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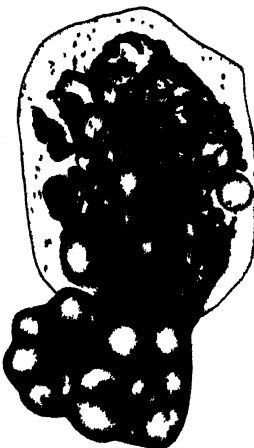
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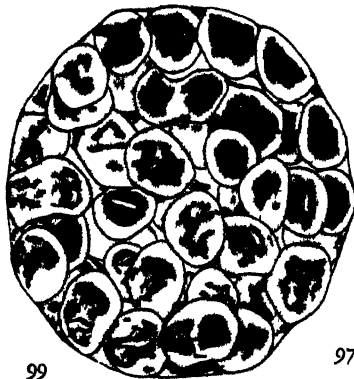
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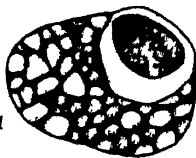
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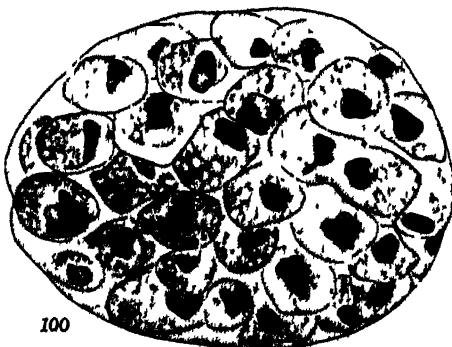
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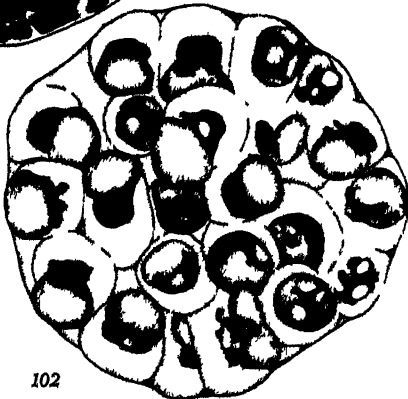
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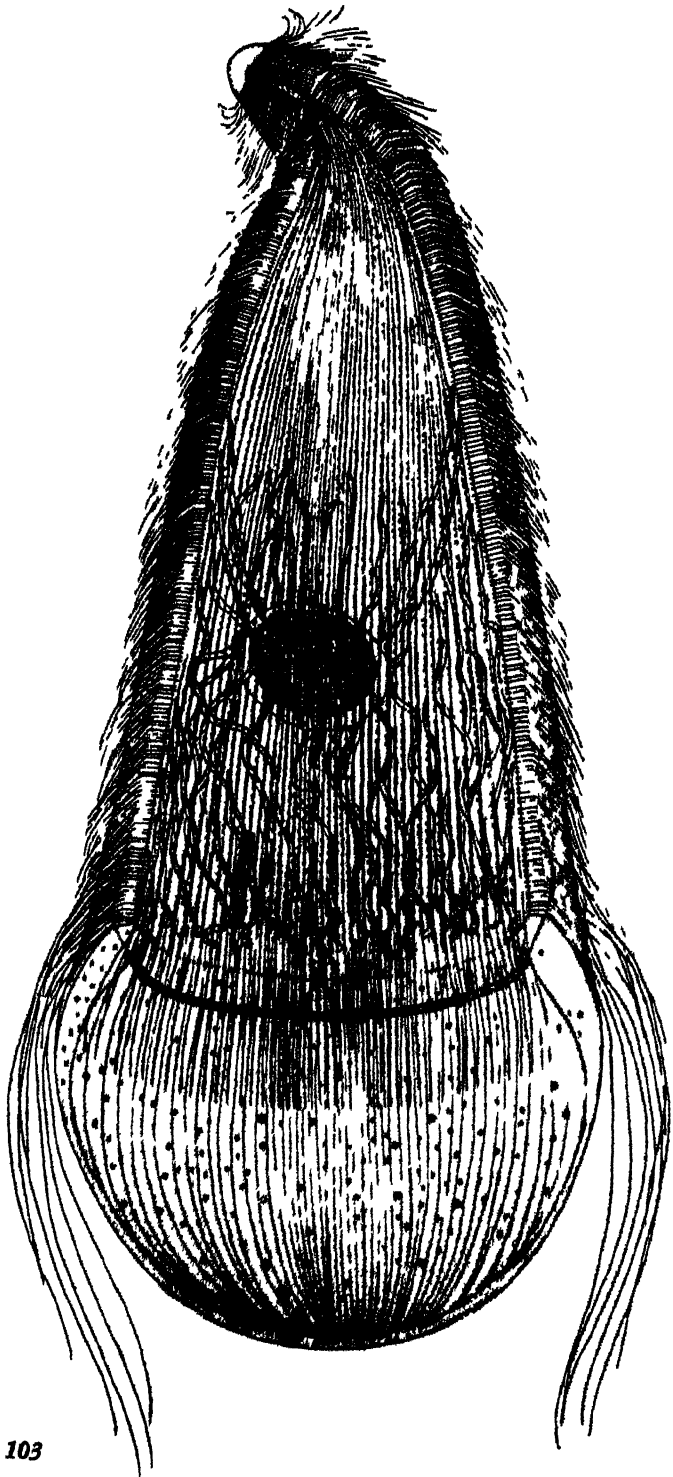
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## PLATE 20

*Trichonympha tulistanica* Bernstein from *Anacanthotermes ochraceus*

Fig 103 The figure is a composite diagram, showing the arrangement of structures that are represented in detail on plates 21 and 22 The aspect shown is a surface view combined in part with optical sections so as to indicate the rostral tube and the thickness of the ectoplasm of the flagellated region The longitudinal plates, both in the rostrum and the body, are shown Over the body region of thick ectoplasm they are omitted, in order not to obscure the picture Detailed explanations of the parts represented accompany the diagrams on the following two plates  $\times 860$



# PLATE 21

*Trichonympha turkestanica* Bernstein from *Anacanthotermes ochraceus*.

Fig. 104. Rostrum and anterior part of body in actual longitudinal section. The anterior end is constituted by the outer cap. Within this is the inner cap, the hemispherical mass of dense-appearing cytoplasm anterior to the crescentic bodies, and one of the crescentic bodies, lying horizontally, seen as a rod across the anterior end of the rostral tube. The rostral tube shows a structure of granules in a matrix substance; the roots of the flagella are attached to these granules. Within the rostral tube is the central rod. The ectoplasm of the rostrum is differentiated into the dense inner, the narrow middle, and the broad outer layers. In the posterior part of the outer layer, between the plates, are peripheral granules. The rostral tube is continuous with the layer of basal granules of the body; the endoplasm is clear within the rostral tube, contains many prenuclear endoplasmic granules posterior to this. The ectoplasm of the rostrum is separated from that of the body by the circular fissure. B.H.  $\times 1325$ .

Fig. 105. Actual section of anterior part of rostrum. The crescentic bodies at the anterior end of the rostral tube are cut across. The central rod is connected no more to one of them than to the other. B.H.  $\times 1325$ .

Fig. 106. The stain is heavy, so that all the material enclosed by the inner cap appears homogeneous and the crescentic bodies cannot be distinguished. An appearance is thus presented like that which led to my erroneous account of the hemispherical blepharoplast surmounting the rostral tube in *Trichonympha*. B.H.  $\times 1325$ .

Fig. 107. Actual cross section of rostrum. Focus on the crescentic bodies, with the central rod, at a slightly deeper focus, between them. Peripheral to the crescentic bodies, also at a deeper focus, are the three layers of ectoplasm and the flagellar plates, which here number 53. B.H.  $\times 1800$ .

Fig. 108. Stereograph showing, as viewed obliquely from behind, the anterior part of the rostrum. In black are the two crescentic bodies and the central rod. Also indicated are the rostral tube and the layers of ectoplasm. B.H.  $\times 1800$ .

Fig. 109. From actual section of body. Group of basal bodies at the innermost ends of the flagellar roots. Because of the heavier stain used, the basal bodies appear as rods, but some show that they really consist of two granules with an interconnecting substance. B.H.  $\times 2700$ .

Fig. 110. Groups of granules showing in place of these rods in material prepared by another technique. Whole mount, Schaudinn, original stain in Heidenhain, retained in protein silver.  $\times 2700$ .

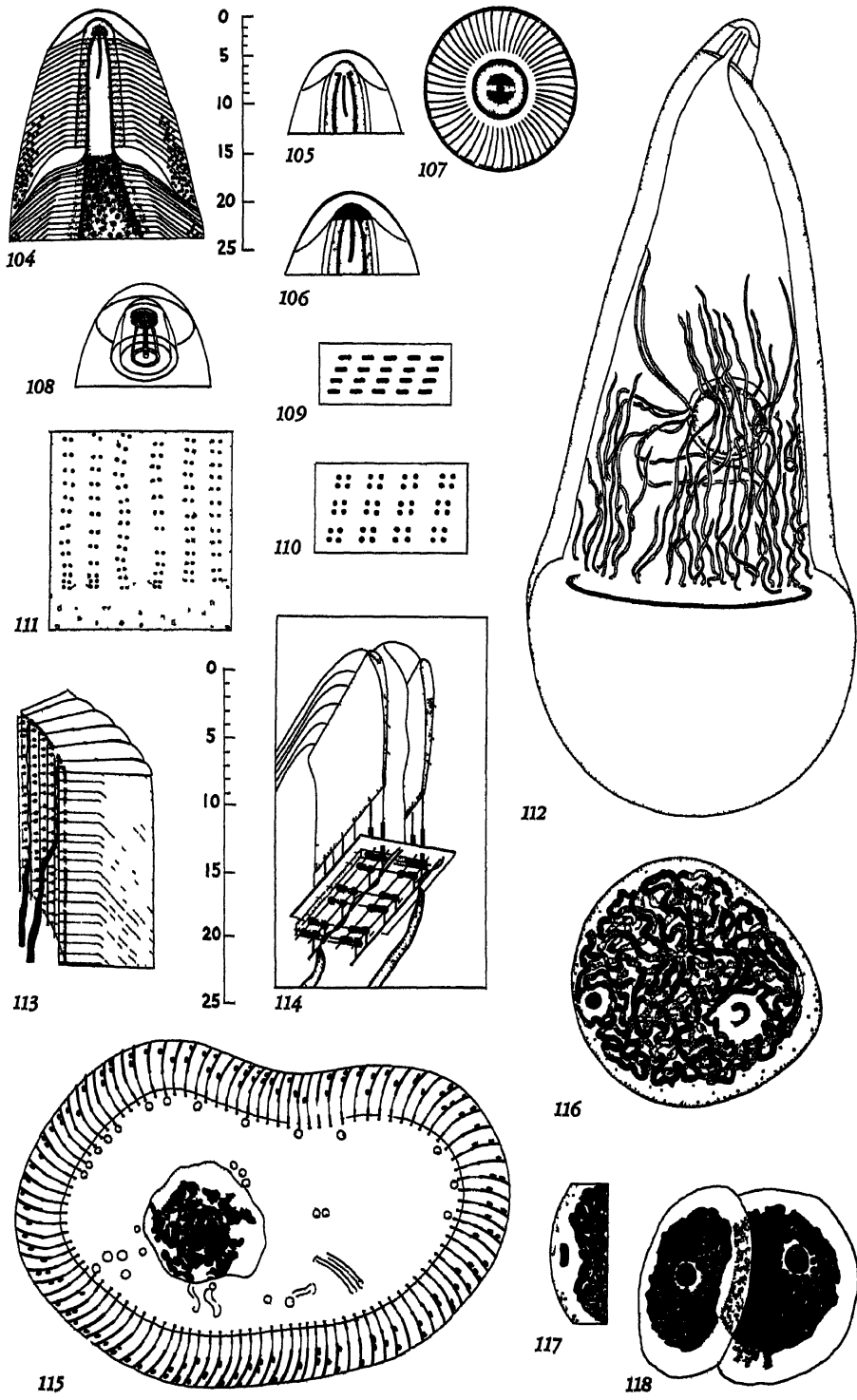
Fig. 111. Part of the body layer of basal granules in the posterior part of the flagellated region, showing the mantle effect of the layer of plasma in which these granules are included. This mantle is marked posteriorly by a sharp, crenulate line. Whole mount, Schaudinn, material retained in protein silver.  $\times 1800$ .

Fig. 112. Whole body showing arrangement of the parabasal bodies and the ring in the endoplasm near the posterior ends of these. The anterior end of each of the stout parts of the parabasals, as shown, lies in the outermost endoplasm, near the layer of basal granules; but each parabasal is extended anteriorly by a parabasal filament. Schaudinn, retained protein silver.  $\times 500$ .

Fig. 113. Diagram of a part of the layer of ectoplasm in the flagella-bearing region, showing the plates and flagellar roots, the layer of basal granules with their plasma mantle, the fibrils in the endoplasm paralleling the rows of basal granules, and the relation of the anterior ends of the parabasal bodies to these fibrils. Certain of the basal granules are connected by short fibrils to this filament. Schaudinn, protein silver.  $\times 1800$ .

Fig. 114. Stereograph showing two flagellar plates and associated structures, the basal granules and their interconnections, and the anterior parts of two parabasal bodies. The flagellar plates are traversed by the roots of flagella, of which the free parts are also shown. Between the two plates is a delicate membrane. The basal granules are in pairs, those of each pair interconnected by a matrix substance forming a rod. The granule at one end of each rod is connected by a short filament to a longitudinal fibril, which lies in the endoplasm just inward to the layer of basal granules. The parabasal bodies come anteriorly

(Continued on page 280)



*Description of Plate 21 (continued)*

into the region of these fibrils, and are continued anteriorly by somewhat sinuous parabasal fibrils. The basal granules are included in a plasma mantle, indicated by two edges in the stereograph. In the innermost layer of ectoplasm the roots of the flagella pass through rodlike enlargements. Across the inner parts of these enlargements is a thin, membranelike layer of plasma. This is indicated in the stereograph as a sheet extending beyond the basal granule mantle.  $\times$  app. 8600.

Fig. 115. Transverse section of the body through the region of the nucleus. The structures in the endoplasm, besides the nucleus, are sections of the parabasal bodies. Between the plates are peripheral granules. B.H.  $\times$  880.

Fig. 116. Nucleus in interphase. Chromatin in a compact mass of coiled strands, rounded nucleoluslike body internal to certain of these strands, curved rodlike body in a clear space peripheral to the chromatin mass. Schaudinn, retained in protein silver.  $\times$  1325.

Fig. 117. The peripheral, chromatin nucleoluslike body has the form of a nearly straight rod. S. protein silver.  $\times$  1325.

Fig. 118. Two closely applied nuclei in an otherwise normal, single, non-dividing *T. turkestanica*. F.R.F.  $\times$  860.

PLATE 22

*Trichonympha turkestanica* from *Inacanthotermes ochraceus*.

Fig. 119. Optical longitudinal section of a strip of ectoplasm, about midway along the flagellated region, where the denser appearing layer terminates. The flagella roots are shown, beginning at the basal granules, which lie just inward to a membranelike structure represented here by a line. The roots pass horizontally through the clear layer of ectoplasm, then bend obliquely posteriorly. Ectoplasmic granules of two types are present: smaller peripheral ones in the outermost ectoplasm, and larger ones in the deeper ectoplasm, as well as more peripherally, posterior to the dense layer. S.H.F.  $\times$  1800.

Fig. 120. Optical longitudinal sections in the same region of ectoplasm as above, showing a fibril that lies between the two plates, above and below it. At this point these fibrils curve outwardly, as they pass posteriorly, from the deeper ectoplasm to the superficial ectoplasm. F.R.  $\times$  1325.

Fig. 121. Surface of the body, in the same region, showing 9 flagellar plates. The anterior, denser layer of ectoplasm terminates posteriorly in the upper part of the figure, and near this termination, between each 2 plates, the fibrils, as shown in figure 120, first appear in the innermost ectoplasm. They then curve outwardly and come in contact, peripherally, with the right side of the plates. Between the plates are shown peripheral granules. F.R.  $\times$  1325.

Fig. 122. Optical longitudinal section of ectoplasm in the same region, showing accurately the relative sizes and distribution of the two types of ectoplasmic granules. The small, peripheral granules are restricted to the outermost layers; they occupy the full extent of the flagellated region of the body, and are present also in the collar. The larger granules are present throughout the ectoplasm, and are restricted to the posterior half of the flagellated region. Two of those figured show binary fission. Part of one parabasal body is shown; its anterior end comes into proximity with the layer of basal granules. It is continued anteriorly by a parabasal filament, which runs close under the layer of basal granules, but that filament is not revealed by this technique. Schaudinn, originally iron-haematoxylin, retained in protein silver.  $\times$  1800.

Fig. 123. Part of surface layer of body midway between nucleus and anterior end, showing the flagellar plates and peripheral granules. F.R.  $\times$  1800.

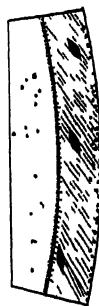
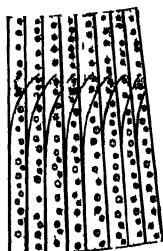
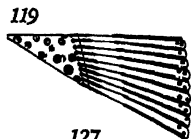
Fig. 124. Part of surface layer of body posterior to the nucleus. The peripheral granules are arranged close to the left side of the plates. Flemming. Slide retained in protein silver.  $\times$  1800.

Fig. 125. Optical longitudinal section of edge of body, showing in the ectoplasm three spindle-shaped parasites and ectoplasmic granules. F.R.  $\times$  880.

Fig. 126. Strip of ectoplasm just posterior to nucleus, with flagellar plates, peripheral granules along the left side of these, and two spindle-shaped parasites between the plates. F.R.  $\times$  880.

Figs. 127-129. Actual transverse sections at different levels of the flagellated zone. Structures shown in all figures are basal granules; a rodlike enlargement around the flagellar roots that meets these granules; a line, representing a thin

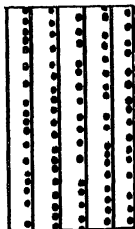
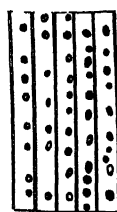
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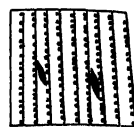
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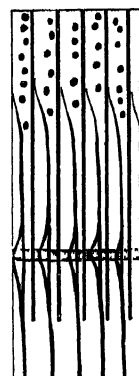
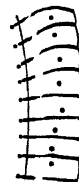
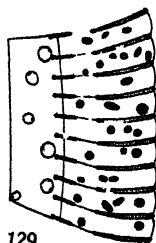


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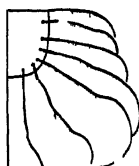
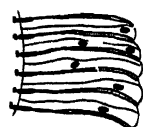


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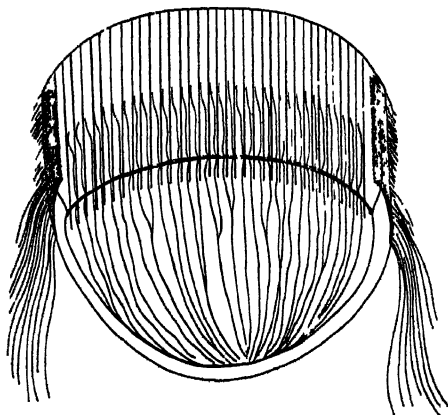
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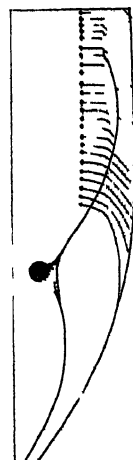
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*Description of Plate 22 (continued)*

membrane, crossing these rodlike enlargements; the fine lines of the flagellar roots in the clear layer of ectoplasm; the flagellar plates in the outer part of the ectoplasm, with a deeper-stained structure at the innermost edge of each plate; the surface ridges between which the plates terminate outwardly. The free parts of the flagella are not shown. B.H.  $\times 1800$ .

Fig. 127. Just posterior to circular fissure. Peripheral granules are present in the outer ectoplasm. Prenuclear endoplasmic inclusions are present in the endoplasm.

Fig. 128. Midway along the flagellated region of the body. Peripheral granules.

Fig. 129. In posterior part of the flagellated region of the body. Ectoplasmic granules of the larger type are distributed through the ectoplasm between the plates. In the endoplasm are cross sections of parabasal bodies; each shows the peripheral parabasal filament.

Fig. 130. Cross section of membranelike structure extending through the whole layer of ectoplasm between the plates. Compare plate 21, figure 114. B.H.  $\times 1800$ .

Fig. 131. Section similar to above, showing the dense and clear differentiation of ectoplasm that sometimes appears on either side of this membrane. B.H.  $\times 1325$ .

Fig. 132. Cross section of ectoplasm showing cross section of a fibril that runs between the plates. B.H.  $\times 1325$ .

Fig. 133. Differentiation of dark and light areas in ectoplasm between the plates. S.H.  $\times 1325$ .

Fig. 134. Surface view of the outer layers of the body in region of the endoplasmic ring, showing the posterior parts of five plates. In the upper part of the figure ectoplasmic granules are shown between the plates. At the right side of each plate a fibril originates, and at the level of the ring it separates into two fibrils, one of which bends inward to the ring. The fibrils then continue posteriorly beyond the flagellated region. F.R.  $\times 1325$ .

Figs. 135-136. Optical longitudinal section in the posterior part of the flagellated region. The ring is soon in section and the fibril, shown in figure 134, bends inward to contact with the ring in 136, close to the ring in 135. F.R.  $\times 1325$ .

Fig. 137. Diagram of the posterior part of the body. In the figure may be seen the short body flagella, the longer posterior flagella, the flagellar plates, the endoplasmic ring, and the fibrils shown in the three preceding figures, bending inward to contact with the ring, continuing posteriorly in the deeper ectoplasm to the posterior end of the body, uniting with one another in places. S.I.F.  $\times 415$ .







# RESULTS OF FEEDING ETHER EXTRACTS OF MALE SUPPLEMENTARY REPRODUCTIVES TO GROUPS OF NYMPHAL TERMITES

BY

E. A. KEENE AND S. F. LIGHT

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# RESULTS OF FEEDING ETHER EXTRACTS OF MALE SUPPLEMENTARY REPRODUCTIVES TO GROUPS OF NYMPHAL TERMITES

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E. A. KEENE AND S. F. LIGHT

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PICKENS (1932) first proposed the hypothesis that an inherent tendency of termite nymphs toward neoteny is inhibited in normal colonies by secretions of functioning reproductives which are acquired by the nymphs through the contacts of colony life. Castle (1934) reported results of preliminary experiments which led him to conclude that "a substance is produced by functioning female reproductive forms which can be removed from their tissues by alcohol or ether, and that this substance when fed to groups of nymphs is capable of slowing down the rate of development of female supplementary reproductives in such groups." Light (1942-1943) has emphasized the variable and somewhat inconclusive results obtained by him in a number of similar experiments and the generally wide range of variation in the rate and extent of development of reproductives in groups of nymphs. He concludes that there is no conclusive evidence for an ectohormonal control, although the existence of such a control is not disproved.

Neither Castle nor Light performed experiments using extracts made from male reproductives, but in the light of what then seemed clearly significant results with female extracts Castle (1934) thought it probable that males also "produce a substance capable of inhibiting the development of gonads of male nymphs associated with them."

Ridder in an unpublished master's thesis (1935; see also Light, 1944) reported results of experiments on extract inhibition of males of *Zootermopsis angusticollis* (Hagen) which if verified would go far toward proving extract inhibition. He held that extracts from functional male reproductives fed to groups of nymphs not only delayed significantly the occurrence of pigmented males, whereas female nymphs in the same groups developed pigmentation at the usual time, but also that egg production was significantly delayed in those groups fed male extracts.

Because numerous more recent experiments have failed to give conclusive proof that inhibition of the development of female supplementaries results from feeding extracts of functional females, it became important to perform an experiment similar to Ridder's as a check on the validity of his findings. Such an experiment was run from August 16, 1943, to November 25, 1943 (100 days). For reasons of convenience *Zootermopsis nevadensis* (Hagen) was used. There seems every reason to believe that results obtained with one species are applicable to the other (Heath, 1927; Castle, 1934; Light, 1942-1943).

The animals from which were developed the supplementary males employed in preparation of the extract were from colonies collected at Inverness, Marin County, California, in May, 1943. The animals used in the experiment itself were obtained from Monterey pine logs brought from the Monterey peninsula, California, in June, 1943, and were from completely inhibited primary colonies. Such colonies contain only one pair of reproductive animals, the founders of the colony; the presence of this pair from the inception of the colony prevents the development of additional reproductives (Light, 1942-1943).

The experimental and control series were made up of 10 groups each. Each group consisted of 30 undifferentiated apterous nymphs, according to the following formula: 6 of the fourth instar, 12 of the fifth instar, and 12 of the sixth instar. Each group was kept in a 4-in. petri dish with a 9-cm. sheet of moist Whatman's No. 1

TABLE 1  
SUMMARY OF RESULTS

Group	Original population VIII-16-43	Final population XI-25-43	Days to male pigmentation	Days to female pigmentation	Days to eggs	Total supplemen- taries
EXPERIMENTALS						
E1.....	30	23	35	56	.	4
E2.....	30	18	42	49	90	3
E3.....	30	18	..	35	56	3
E4.....	30	25	21	63	63	5
E5.....	30	19	49	56	..	3
E6.....	30	23	28	28	56	7
E7.....	30	21	76 <sup>a</sup>	36	49	2
E8.....	30	23	91 <sup>a</sup>	35	56	3
E9.....	30	20	49	35	56	4
E10.....	30	24	42	28	56	5
Average.....	30	21	48	42	60 <sup>b</sup>	3.9
Range.....	30	19-25	21-91	28-63	49-90	2-7
CONTROLS						
CE1.....	30	18	49	35	56	5
CE2.....	30	18	90	35	42	5
CE3.....	30	24	77	35	42	5
CE4.....	30	12	63	90	.	2
CE5.....	30	24	28	90	56	3
CW1.....	30	16	49	28	49	3
CW2.....	30	22	77	21	56	2
CW3.....	30	24	56	28	56	4
CW4.....	30	22	35	35	56	4
CW5.....	30	19	35	42	70	4
Average.....	30	20	55.5	44	53 <sup>b</sup>	3.7
Range.....	30	12-24	28-90	21-90	42-70	2-5

<sup>a</sup> Reproductive soldiers.

<sup>b</sup> Omitting those not producing eggs.

Filter Paper which served as food on which the extract was administered to the experimentals.

Five groups of the control series (CE1-5) were fed papers which had been soaked in ether and dried; the remaining controls (CW1-5) were fed papers which had been moistened with distilled water. It will be seen from table 1 that there was no significant difference in reproductivity or viability between these two series of controls. The controls are therefore considered to constitute a single series and the results are so treated in the discussion.

Extract was prepared as needed, approximately every two weeks, following the methods of Ridder. Twenty pigmented functioning male supplementaries were ground in a mortar with 40 cc. of anhydrous ether until the animals were well macerated. The solid matter was allowed to settle and the extract decanted and placed in a closed flask. Some was used immediately, and the remainder kept until the next week, since the animals were fed at weekly intervals. One filter paper was saturated with 2 cc. of the extract for feeding each experimental group. It was then allowed to dry thoroughly and was moistened with water before being put into the petri dish containing the termites. If all termites had lived and each had consumed an equal amount of the extract-bearing paper, all of it being consumed during the week, each nymph would have received daily 0.004 of the extract of one male supplementary. Actually of course, the paper was by no means entirely consumed, the numbers of animals declined, and there is no way of estimating the individual dosage.

Every few days a few drops of water were added to the paper in each dish. All groups were examined once a week, the census was taken, records were made of individuals showing pigmentation, eggs present were recorded and removed, and any signs of disease were noted. At this time also fresh papers were supplied and fragments of old papers and fecal matter were removed. The termites were of necessity removed from the petri dishes during this process.

Each group of both the experimental and the control series gradually decreased in population until, at the end of the experiment, the population in the experimental groups averaged 21 (see table 1) and that of the controls averaged 20. A similar decline in population of small groups has been found unavoidable under experimental conditions (Light, 1942-1943). Inspection of the detailed record shows that the weekly decline in population by groups was, in general, similar for experimentals and controls. A general decrease at the beginning was probably a result of the handling and disturbance incident to setting up the groups. No group in either series died out completely, and there is no indication of especially unfavorable or differentially unfavorable conditions in either of the series.

Shortly after the groups were established, there was found to be present a fungus which caused brown spots to appear at the ends of the appendages and on the bodies of the animals. Although a few deaths were apparently caused by this parasite, the incidence of the disease decreased. No differential was to be observed in the incidence of fungus attack between individuals and groups of the experimental and control series. A scatter diagram showed no positive correlation between high incidence of fungus attacks and low final population. There is no indication either from the final censuses (table 1) or from weekly changes in the populations of the individual groups that the decrease in population had any relation to the incidence of the disease or that the decrease was significantly different between experimentals and controls.

The time required for the appearance of pigmented males in the experimental groups, fed an ether extract of functional male supplementaries, averaged 48 days, with a variation from 21 to 91 days (see table 1). In the control groups, fed no extracts, the average time required for the appearance of pigmented males was actually longer, 55.5 days, with a range of 28 to 90 days. Clearly there is no evidence here for an inhibiting influence of extracts of males.

Pigmented females were recorded in the groups of the experimental series in an average of 42 days, with a range of 28 to 63 days. In the controls the average time was 44 days, with a range of 21 to 90 days (see table 1). No evidence was obtained, therefore, for a delaying effect on female pigmentation from the feeding of extracts

of males. In this particular our findings agree with those of Ridder. But the great difference between males and females of extract-fed groups with respect to time of incidence of pigmentation reported by Ridder is not confirmed by us.

The length of time required for the appearance of the first egg will be seen to have varied also. In those groups of the experimental series which produced eggs, the time to the first egg averaged 60 days, with a range of 49 to 90 days, and in the controls, 53 days, with a range of 42 to 70 days (see table 1). For three groups, E1, E5, and CE4, no eggs were recorded in the entire 100 days, although pigmented females were present in all three of these groups at the end of the experiment. In arriving at the averages given, these three groups were disregarded. If we consider these groups as having required the full 100 days, the averages become, for the experimentals, 68 days, and for the controls, 58 days. Here the average findings as well as the ranges might seem to offer some slight evidence for an inhibiting effect but nothing comparable to that supposedly obtained by Ridder. Actually the series are not large enough to make these differences really significant. One group in the control series produced no eggs. The occurrence in the experimental series of two such groups and one group in which egg laying was long delayed is probably of no significance.

At the end of the experiment all pigmented females were opened and fixed in Bouin's fluid. After fixation the seminal receptacles were removed, stained in Harris' hematoxylin, sectioned, and the sections studied to determine the presence or absence of spermatozoa in the receptacle. The presence of spermatozoa in the seminal receptacles of the females of the control groups and their absence from the females of the experimental groups would furnish contributory evidence for an inhibiting effect of the extract.

When the sections of the seminal receptacles of the pigmented females were examined, it was found that in 9 of the 10 groups of the experimental series one or more pigmented females contained spermatozoa, indicating that the feeding of male extracts had not prevented sexual functioning of males in these groups. The one experimental group (E3) in which none of the pigmented females contained spermatozoa was the only one in which no pigmented male was recorded (see table 1). The incidence of spermatozoa in the pigmented males of the experimental series was strikingly similar to that of the control series. Of 19 females examined in each series, 7 in each series were without spermatozoa and 12 with spermatozoa.

## DISCUSSION

Ridder reported a far greater uniformity in time of occurrence of pigmentation of both males and females than has been reported by others (Light, 1942-1943). In the results of the present investigation in which Ridder's experiments were repeated in essential features, the uniformity characteristic of his results is conspicuous by its absence. Both experimentals and controls showed a wide variation in the length of time required for the appearance of pigmented males (see table 1). When averaged, the time required proved to have been less for the experimentals (48 days, range 21-91) than for the controls (55.5 days, range 28-90). According to Ridder's results, the time required for male pigmentation was, for the experimentals, 74.4 days (range 55-96), and for the controls, 23.9 days (range 21-29)! On the average, the male nymphs fed extract in the present experiment became pigmented somewhat sooner than the controls which did not receive the extract! The difference in this respect between controls and experimentals in our experiment can hardly be significant, since the numbers of groups is small and the range is great. However, there is certainly no evidence of inhibition of male pigmentation.

The average time required for pigmentation of the female nymphs in Ridder's experimental groups was 23.8 days (range 15-43), and in the controls, 22 days (range 19-20), thus indicating no effect from their having consumed the male extract. In the present experiment, the experimental groups required an average of 42 days (range 21-63) for pigmentation and the controls 44 (range 21-90). Our

TABLE 2  
OCCURRENCE OF SPERMATOOA IN PIGMENTED FEMALES

Group	Presence or absence of spermatozoa	Group	Presence or absence of spermatozoa
E1.....	0 +	CE1 .....	0 0 0
E2.....	+		+
E3.....	0 0 0	CE2 .....	+
			+
E4.....	+	CE3.....	+
	+		0 0
E5.....	+	CE4 .....	0
	0	CE5 .....	+
E6.....	+	CW1.....	+
E7.....	+		+
E8.....	+	CW2.....	+
	+		
E9.....	+	CW3.....	0 +
	0		
E10.....	+	CW4.....	+
	0		
	+	CW5.....	+

results agree with Ridder in that there is no indication of inhibition. In both our series, however, the range of variation was very much wider than that reported by Ridder.

Ridder found eggs in his control groups in an average of 31.1 days (range 29-36), and in his experimentals in an average of 94.7 days (range 80-102) ! In the present experiment, egg production in the controls required an average of 53 days (range 42-100, including one which produced no eggs) and in the experimentals the average time was only 60 days (range 49-100, including two which produced no eggs). Although a slight delay is suggested in the time required for egg laying in the experimental series, it is not comparable to that reported by Ridder, and a variation from 42 to 100 days for all groups seems to indicate that there is no significant difference between the two groups in the time required for egg production.



Here again the wide range of variability is in sharp contrast to that reported by Ridder, especially that for his control series, 29 to 36 days, a range of only 7 days!

Ridder reported a positive correlation between delay in egg production and delay in male pigmentation and assumed that the former condition was a result of the latter. More recent work has shown, however, that parthenogenesis is readily accomplished by segregated female termites and that the laying of eggs does not necessarily mean that copulation has taken place (Light, 1942-1943), although there seems good reason to believe that copulation does normally occur if males are present (table 2).

It is generally accepted (Snyder, 1915; Castle, 1934) that increased pigmentation is a sign of sexual maturity in termites. However, increase in pigmentation is gradual, and consequently it is uncertain exactly when an individual is mature and sexually functioning. In order to determine whether or not those males which did not become pigmented were actually functioning, the seminal receptacles of the pigmented females in the groups were sectioned and examined for spermatozoa. It will be noted (tables 1 and 2) that, except for one group (CE4) in the control series, spermatozoa were found in the seminal receptacle of at least one of the females in each of those groups in which there was a pigmented male. This would seem to indicate that a male in each such group had copulated, presumably the one noted as conspicuously pigmented.

The question may be raised of the possible effect of the differences in size of the groups used by Ridder and those of the present experiment. Ridder used 50 animals per container, whereas in this investigation the groups consisted of only 30 nymphs, a limitation imposed by the size of the colony from which the animals were taken. There is no reason to believe, however, that size difference of the groups alone could explain such a marked difference in results. Light (1942-1943) reports a general similarity of results in a wide range of experiments in which the numbers of individuals varied.

A possibility of error is introduced in the grinding of the whole insect in preparation of the extract. Conceivably two antagonistic substances may be present in the reproductives, one influential in promoting sexual development, and the other inhibitory. It is possible that both of these might be extracted in a maceration of the whole animal and tend to offset each other. This, of course, would make Ridder's findings all the more surprising. The method of obtaining the extracts as well as the use of ether for extraction represents an attempt to follow Ridder's procedure as closely as possible. In this connection it should be recalled that Light (1942-1943 and 1944) has obtained results, not greatly different from those of the present experiment, from feeding surface extracts of females and also extracts of females made with water and with various alcohols (70 per cent ethyl, absolute ethyl, and methyl) and with benzine (petroleum ether). However, the over-all trend of the evidence indicates a slight inhibitory effect from feeding extracts of female supplementaries.

## SUMMARY AND CONCLUSIONS

1. Two series, of ten groups each, of nymphs from a colony of *Zootermopsis nevadensis* (Hagen) were isolated at the same time and were given the same treatment except that one series was fed an ether extract of functioning male supplementary reproductives of the same species, and the other series received no extract. Each group consisted of 30 nymphs and included nymphs of several different instars in the same numbers.

2. The relatively low death rate and the relatively high production of supplementaries and eggs indicate that the reproductivity of the groups was not influenced by disease to any significant extent.

3. No significant differential was observed between experimentals and controls with respect to mortality or time of deaths.

4. Observed occurrence of supplementaries in the experimentals and control series gave no indication of delay in development of supplementaries in the experimental series which could be interpreted as an inhibitory effect of feeding the extracts of males. Pigmentation of male nymphs was not delayed in the experimental groups fed male extract. Pigmentation of female nymphs was likewise not delayed in the experimental groups. About the same length of time was required for the appearance of pigmentation in the two sexes both in experimental and control series. Finally, no significant difference with respect to the length of time required for the appearance of the first egg was observed between experimental and control groups.

5. The usual differences between groups with respect to these features reported by Light (1942-1943) were found in both series. The time required for the occurrence of the first pigmented male varied from 21 to 91 days in the experimental groups and from 28 to 90 days in the controls. The experimental groups required from 28 to 63 days for the appearance of the first pigmented female, and the control groups, from 21 to 90 days. The experimental groups producing eggs required from 49 to 90 days for the appearance of the first egg, and the controls, from 42 to 70 days.

6. In view of the fact that these results are in agreement with the results of the extensive series of experiments carried out by Light (1942-1943 and 1944) on supplementary development in general (reproductivity) and the results of feeding extracts of female supplementaries, it seems necessary to disregard Ridder's reported findings and to consider that no actual evidence exists for extract inhibition of male reproductivity.

7. The inhibition theory still seems the most available one, but evidence for the existence of an ectohormonal substance is lacking for the male and not conclusive for the female.

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# A STUDY OF SPONTANEOUS MUTATION

BY  
RICHARD B. GOLDSCHMIDT

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# A STUDY OF SPONTANEOUS MUTATION

BY

RICHARD B. GOLDSCHMIDT

## I. INTRODUCTION

IN 1935 an interesting spontaneous outbreak of "mutation" was discovered which seemed to belong to the group of mass mutation. A few preliminary notes have been published on the findings; but although they give a correct general description of the facts, they also include errors in the analysis of results based on incomplete data. Personal circumstances have twice enforced long interruptions of the thorough analysis since begun, and consequent repetitions of the experiments. Publication of the facts *in extenso* now seems justified, since the happenings appear to be of basic importance for an understanding of the phenomenon of mutation.

Though natural, spontaneous mutation is the most important phenomenon of genetics, upon which the whole structure of classic genetics is built, our knowledge of it is nevertheless meager since hardly any systematic effort has been made to understand it (see, however, Baur, 1918). Mutants are usually picked up in the field, the pen, the bottle. Sometimes they are derived from pedigreed broods, and it is known how many gametes have mutated and at which point in the germ-cell cycle. But this is about the limit of information available, though certain features ought to have diverted research toward more detailed observations. Looking over the old literature on *Drosophila*, one finds, for example, such data on the origin of standard mutants as the following: Truncate was obtained in Beaded stock; both balloon and vestigial from truncate; purple from vestigial; kidney from vestigial and purple (N.B.: kidney frequently reappears in pure inbred vestigial cultures!); ebony from balloon; rough from truncate; spread from Beaded and vestigial; cardinal from vestigial  $\times$  wild; plexus from spread. Here eleven standard mutations have a common pedigree. Other examples are: bow from rudimentary  $\times$  wild; Bar from the same, as also blistered and jaunty; curved also was obtained from rudimentary. A great many standard mutants appeared in crosses of other mutants: Sepia from Lobe  $\times$  dachs; Dichæte from cleft  $\times$  sable forked; bithorax from maroon  $\times$  wild; ski from white  $\times$  wild; hairless from pink spineless  $\times$  telescope; divergent from Star  $\times$  telegraph; hairy from singed  $\times$  inflated forked Bar; black from miniature  $\times$  wild; gap from black  $\times$  are; dachs from sable  $\times$  wild; comma from dachs  $\times$  pink; morula from peach  $\times$  wild; fringed from jaunty  $\times$  white; etc.

All this may be a matter of chance, based upon the unintentional selection of experimental procedure and completely insignificant. It may, for example, be owing to the fact that mutant stocks and crosses were watched more carefully. But it is equally possible that a rule is hidden behind these facts and that a systematic study of spontaneous mutation will show that mutation is not haphazard.

A small but significant group of facts (aside from the important facts of radiation genetics) is available to show that mutation may occur occasionally as a mass phenomenon. The cases thus far made known (Demerec, Plough-Holthausen, Spencer, Valadares, Goldschmidt) have been discussed by me previously (Goldschmidt, 1940). Since that time new cases that have been more closely followed than the earlier ones have been reported by Neel, and by Tiniakoff; and Demerec

has recently announced (1943) that he has found yet another case. Thus far none of these have been analyzed in such a way as to make it possible to state with certainty what had actually happened. Since more details are known in the case to be described in this paper, it seemed advisable to put the data on record and to show that mutation might be something which is concerned not merely with the locus in question. In the course of this work numerous side lines connected with it had to be taken up. Some of them have been included here, though they might have been published separately, because I thought there should be on record at least one case in which the material used had been thoroughly tested. They might not seem very important if viewed on their own merits; yet as part of the whole they should, I thought, be included. In spite of much work not everything could be cleared up completely. It will be easy to suggest further experiments which should have been made at one point or another. But any piece of work involving innumerable details must one day be regarded as finished for the time being; and though I am conscious of the gaps still existing in the present work, I thought that this moment had now come.

#### ACKNOWLEDGMENTS

In the course of the years since 1935 I have been favored with much assistance. During 1936-1937, while my work was being transferred from Berlin to Berkeley, where all laboratory facilities had to be created afresh, Professor Curt Stern, of the University of Rochester, kept my stocks of *Drosophila* and returned them to me intact. I am deeply grateful for this kindness. The Department of Zoölogy of the University of California and its entire staff did everything possible, in an overcrowded building and within the means allowed by a not too plentiful budget, to make room and supply equipment for myself and for the research group which was to be built up. I am under great obligation to my colleagues who made this possible even at the cost of personal sacrifices. The Committee on Research of the University has allocated to me, year by year, funds for research assistants, and has repeatedly granted extra funds for special needs. I beg to express my gratitude to this Committee and to President Robert Gordon Sproul and Vice-President Monroe E. Deutsch, to whom I never appealed in vain. While the WPA organization was in existence, my work and that of my group of collaborators was accepted as a WPA project, and all the technical help needed was furnished freely—assistance for which I shall always be grateful to this organization and to its Supervisor for the Biological Sciences, Dr. R. Stohler.

The junior authors of the present paper have all served at some time during these years as research assistants<sup>1</sup> and have shared in the experiments, especially in repeating on a larger scale the findings of the senior author. He alone assumes the responsibility for the work, especially the decisive experiments, and for the presentation of the results. Mr. Masuo Kodani and Miss Aloha Hannah did all the cytological work. No result was accepted which could not be seen identically by both the senior author and the assistant, the former alone taking the responsibility for the data. Mrs. Richard M. Eakin kindly contributed the statistical work, of which only a part is incorporated in this paper. We are much obliged to Mrs. Laura G. Rauch for editing the manuscript.

<sup>1</sup> Richard Blanc is now at the University of Rochester; Ruth Fields, at the University of British Columbia; Claude Vilee, at the University of North Carolina; and Werner Braun is now with the Division of Veterinary Science, Department of Agriculture, of the University of California.

## II. THE STARTING POINT

A stock bottle of plexus flies started from the original Columbia stock seven years before and never appearing to be different from the standard type was found to contain (winter, 1933-1934) a majority of flies very different from the typical plexus as found in the other bottles. The venation showed an extreme type of plexus formation far beyond that of the mutant plexus (details will be given further on). Also, about half the females and a few rare males were blistered. In the great majority of individuals this blister (filled with fluid in the young flies and covering a considerable part of the wing, which is frequently torn when the blister breaks) is found on one wing only. I was originally interested in this problem of asymmetry and began inbreeding and selecting this stock in many different ways. An account of some of this work will be presented below. (Among the Pasadena stocks a line exists, listed in *DIS* as px bs seh, which is phenotypically very similar to the stock described. A note from the Pasadena stock keeper says that it is probably an allele of bs blistered. We shall see that the bs-locus is also involved in our line.) The line, which will be called plexus blistered in a purely descriptive sense, was controlled through many generations of brother-sister matings and remained constant. It has now been bred for about ten years. It has remained constant except for the features to be described, but in later years some of the characteristics of blistering became variable (details below). In a series of brother-sister matings in 1934-1935, 134 matings gave 10,795 ♀, 10,199 ♂. Among these were found, besides a number of individuals with half thorax or with abnormal abdomen—conditions which did not seem to be inherited—the following atypical individuals:

9 ♂	one wing like rudimentary or dumpy	1 ♂ dwarf
2 ♀, 2 ♂	spread wings	1 ♂ beaded
1 ♀, 2 ♂	shortened broad wings	

It will be noted later that these types, which did not seem to be inherited in simple tests, are all represented among the genuine mutations derived from this stock. They appeared in these lines after the manner of mutants, but were not isolated at that time because the work was originally not concerned with mutation. In the stock bottles no such types have been found.

On December 8, 1934, a pair from a closely inbred plexus blistered stock (abbreviated px bl, which does not signify any locus) was mated, a female with blisters on both wings and a male without blisters. The offspring were (besides the extreme plexus always present and therefore not mentioned):

No. 4100 B <sup>1</sup>	53 ♀	46 ♀ one wing blistered, 2 ♀ both wings blistered
	103 ♂	2 ♂ blistered

A second generation was bred from both females with both wings blistered and the two blistered males (nos. 4302 and 4303):

No 4302 B	77 ♀	40 ♀ blistered, 2 ♀ both wings blistered
	72 ♂	2 ♂ blistered

This resembles the foregoing generation except for the small number of males. (See later discussion of sex ratio in px bl.)

No. 4203 B	5 ♀	px blistered, 4 ♂ px 1 ♂ hemithorax
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<sup>1</sup> B signifies work done in Berlin, 1934-1936. Record numbers of later work begin again with no. 1.

There had frequently been sterile pairs, but never a semisterility of this type. (The parents left in the bottle were still alive at the time of hatching.) Furthermore, never had all females been blistered. Otherwise these individuals were typical plexus blistered. From these a third generation was bred. One fertile pair (the only available virgin ♀) gave:

No. 4474 B    136 ♀ and 70 ♂ wild type (no plexus or blistered)  
                   18 ♀ and 19 ♂ low-grade plexus like the typical mutant plexus  
                   1 ♂ rudimentary

Again the parents were still in the bottle when the first count was made. These numbers are perfectly regular. They suggest that about  $\frac{7}{8}$  of the females and about  $\frac{3}{4}$  of the males were normal;  $\frac{1}{2}$  of the wild-type males were missing,  $\frac{1}{8}$  of the females and of the potential males (if the sex ratio were normal) being typical plexus, and a single individual was rudimentary (turning out to be an allele of *r*). This regularity, together with the presence of the parents and the further behavior of the line, excludes any error. No contamination is imaginable which would give this result. (See below.) It suggests that something involving an autosome and the sex chromosome (ratio 7:1,  $\frac{1}{2}$  of the males lethal) had occurred, which resulted in the disappearance of whatever it was that caused the extreme plexus and the blistered type in one combination, and the disappearance of the plexus type, at least phenotypically, in the rest. The rudimentary male might be an ordinary mutant at this locus, as such a mutation is said to be rather frequent, though it never occurred in my stocks (for a single recurrence see below). The following facts will show that this is not the case. *I wish to emphasize again the fact that all the offspring of the plexus blistered pair derived from a long constant pedigree were different from the parents.*

The rudimentary male could not be mated successfully. A fourth generation was bred from wild-type as well as from plexus flies, with the result recorded in table 1. The data show that some of the wild-type females and males were true-breeding (later extracted). Some females were heterozygous for rudimentary, producing less than  $\frac{1}{2}$  rudimentary males (rudimentary is less viable.) Another wild-type female was heterozygous for rudimentary in one X chromosome and for a new type with pointed wings in the other, which turned out to be a new allele of silver. Some of the females of this brood 4612 showed an indication of pointed wings (slight dominance effect). The combination of two plexus flies produced a typical plexus blistered brood with a relatively small proportion of blistered females, but with reappearance of the strong plexus type of venation lost in the foregoing generation. The changes, then, had begun with a "return mutation" to plus<sup>xx</sup> in the gametes of *px bl*, an event which somehow simultaneously released pointed and rudimentary. It may be anticipated that *px bl* contains also a mutant condition at the *bs* locus. This also had disappeared, and a male lethal had appeared.

It is to be emphasized that neither the rudimentary nor the pointed wing, which is also a pure-breeding sex-linked type (actually a silver allele), could have arisen by ordinary mutation. As some of the wild-type females were heterozygous for one or both, a mutation ought to have occurred in the sperm of the grandfather, who could have sired neither a rudimentary son nor a daughter heterozygous for both pointed and rudimentary in different X chromosomes (except by double mutation

and male crossing over). Also, three out of four wild-type daughters would have had to be sired by sperms with the rudimentary mutation. Furthermore, there is the nonconforming rudimentary male (among 90) in  $F_3$ .

The details of the following generations belong to the special analysis of the types produced and will be found in the respective sections further on. It turned out that the wild-type individuals could be either pure-breeding or heterozygous for rudimentary, pointed, or typical low-grade plexus in different combinations. (The bs allele was absent.) The decisive point is that a true-breeding wild type was established that was not distinguishable from any other. The pointed wing was easily isolated as a sex-linked recessive located at the left of the X chromosome. It is an allele of silver and it has bred true for years. The rudimentary type also was isolated. It was homozygous, heterozygous, or neither, for something producing plexus, and it could also contain pointed. It behaved as an allele to standard rudi-

TABLE 1  
OFFSPRING OF 4474

No.	Parents 4474 <sup>a</sup>	+		Pointed ♂	Highgrade px of original type		px blistered ♀	Rudi- mentary ♂	
		♀	♂		♀	♂			
4609 B	+ × +	148	170	..	..	..	..	..	
4610 B	+ × +	185	80	..	..	..	..	37	
4612 B	+ × +	151	..	65	..	..	..	55	
4613 B	+ × +	8	3	..	..	..	..	1	
4614 B	px × px	...	..	..	72	67	13	..	1 dwarf

mentary and showed the same peculiarities (like female sterility). In stocks over double yellow it bred true, but frequently changed the phenotype (independently of external conditions) between medium-sized, dumpy-shaped wings and very short ones and other types (see below). The viability decreased with shortness, and the extremes produced only female offspring by inviability of the males, all transitions existing. The plexus blistered type recovered from the lines described above bred true to this type and was obviously again the old plexus blistered stock, thus showing that everything had happened within this stock. The type with a moderate amount of plexus was again heterozygous for pointed or rudimentary or both. Or it bred true, and has remained so for years, never producing a blistered or high-grade px fly. It behaves in tests with standard plexus as if it were identical with it. We point to the one dwarf (not producing offspring) in  $F_4$ , as dwarf reappeared again.

In one of the lines derived from the original abnormal brood other genetic types appeared. From the brood 4612 (see table 1), which had yielded normal females and pointed and rudimentary males, a further generation was bred from a normal female with a rudimentary male (no. 4764 B). It produced:

76 ♀ +	25 ♀, 15 ♂ low-grade plexus, not pointed
33 ♂ pointed	29 ♀, 32 ♂ rudimentary

The mother, then, was heterozygous for rudimentary; but only  $\frac{1}{4}$  of the daughters, though nearly  $\frac{1}{2}$  of the sons, were rudimentary. The other X of the mother must

have contained pointed, and accordingly  $\frac{1}{2}$  of the males are pointed. Furthermore, both parents must have been heterozygous for plexus, which, however, did not show the recombination with pointed (it was not checked for rudimentary)—but this has no special meaning, as only some alleles of pointed can be clearly distinguished in the presence of plexus, which tends to make the wing tip appear blunted. Also, about half of the expected males were missing. A pair of plexus flies from the foregoing brood was mated (no. 4988 B), with the result:

62 ♀, 35 ♂ plexus like mother	.. 24 ♂ rudimentary
9 ♀, 7 ♂ plexus and pointed	1 ♀, 3 ♂ plexus, soft spread blistered

Here, first a plexus and pointed phenotype occurs in a ratio among the plexus flies which looks like 7:1, and the mother was heterozygous for rudimentary, as expected, though the number of  $r \text{ ♂}$  is deficient. A new type appears in a few individuals, exhibiting the same plexus as the parents and soft spread blistered wings. (Again we anticipate the genetic constitution of these flies. A full discussion of the entire story with additional facts will be given in a later section. These flies are homozygous for *px* and for a pointed allele, called *svr<sup>poi</sup> b<sup>1</sup>*. Furthermore, they are homozygous for a second chromosome recessive at the *arc* locus, to be described as broad angular (*bran*). The combination *bran* and standard pointed produces what will be described later as soft blistered flies. The soft spread blistered type, however, is the result of recombination of *bran* with a new allele of *poi*, *poi* blist (*svr<sup>poi</sup> b<sup>1</sup>*), which thus arose simultaneously with *bran* (see full discussion later). The *px* pointed phenotype turned out to be heterozygous *bran* with the new pointed allele.

These, then, were the original happenings anticipating part of the interpretation which will be presented further on. To enumerate the main features:

1) A standard plexus stock had changed in the stock bottle into the *px bl* type, which turned out to be mainly a combination of *px*, different *bs* alleles, a sex-linked locus connected with blistering, and autosomal modifiers for the degree of plexation.

2) In a large set of pair breedings from *px bl* for purposes of selection the entire offspring of a single pair was spontaneously different from the parents. In ordinary genetic language, the happenings would have to be described in the following way:

a) The original homozygous low-grade plexus which had disappeared as such in the formation of the high-grade plexus blistered stock from *px* had reappeared as true-breeding plexus (without *bs*).

b) This plexus mutant locus, as well as *bs*, had completely reverted in other flies, producing a true-breeding wild type.

c) The parental plexus blistered type which was absent in  $F_1$  was recovered in  $F_2$  and again bred true.

d) A new type with pointed wings was produced which turned out to be an allele of silver (*svr<sup>poi</sup>*).

e) A new type of rudimentary was produced, indistinguishable from the standard mutant of this name and combined or not with plexus blistered and *svr<sup>poi</sup>*.

f) A weak type with soft spread blistered and plexus wings segregated later, which turned out to be a recombination of *px*, another *svr* allele *svr<sup>poi</sup> b<sup>1</sup>*, and an arc allele named *bran*.

g) Sex-linked lethals and strange ratios were present.

When these facts were first determined we tried to check on the possibility of an

experimental error, as the change of all the progeny from the type of the parents is an event to be looked upon with suspicion. It has already been mentioned that the parents were still in the bottle when the offspring started hatching. But let us suppose that this does not exclude contamination. From the results obtained, such contamination would have had to be with a wild-type fly. Further, one must suppose that pointed, rudimentary, pointed blistered, and bran were already present in the px bl stock but remained invisible until freed from px by a cross. What we considered to be the offspring of px bl flies would then have to be at least grandchildren of the supposed contamination, with the first contaminated generation looking like px bl. It would also have to be assumed that two alleles of silver and bran were hidden in the grandparents without showing their well-known effects, in addition to rudimentary in a male X without showing it, etc. This is more than the worst pessimist could expect.

Actually, genetical analysis of the px bl stock, which was started at once, never betrayed the presence of  $svr^{poi}$  or bran or rudimentary in the stock. But the decisive point is that all these mutants were later obtained many times from px bl as well as from other pedigreed derivatives of this stock, which obviously is likely to produce these mutants (and also others) under certain conditions. It is to be regretted that a complete repetition of all the features just described in the offspring of a single pair never occurred. But the same upheaval occurred again in stock bottles. Moreover, the individual steps were found over and over again in controlled matings either of px bl or its derivatives, e.g., reverse mutation px to plus and again plus to px, plus to different  $svr^{poi}$  alleles, or one allele into another, alone or simultaneously, with mutation to bran or one of its alleles, return mutation from both to plus, mutation at the bs locus, aside from a relatively high frequency of other mutants. The details, to be described below, completely exclude any idea of experimental error.

### III. ANALYSIS OF THE px bl STOCK AND THE MUTANTS IN THE REGION OF THE PRIMARY CHANGES ( $svr$ , px, bs, a)

Whatever may be the genetical basis of the briefly described primary mutational changes, the only hope for gaining an insight is to elucidate as completely as possible the genetic constitution of the original px bl line and of the stocks of mutants at the loci found to mutate rather frequently, whether they are derived from the original line directly or indirectly. We propose to present first the data for the mutants and afterward those for the px bl stock.

#### 1. THE BASIC RECURRENT MUTANTS

##### a. THE MUTANT pointed, $svr^{poi}$ (ABBR.: poi)

*Phenotype.*—Pointed looks like an exaggerated silver. It is still paler (comparison with two Pasadena stocks) and the wings are more pointed. In  $svr$  the pointed wing is not visible in mass cultures and not very distinct in pair cultures, though it comes out better when  $svr$  is extracted from crosses. In  $svr^{poi}$  it is always clear, more extreme in the females and also more typical in pair cultures. The body color is best described in comparison with others (as overleaf).<sup>a</sup>

<sup>a</sup>I owe this description to my former assistant, Dr. C. A. Villee, I myself being partly color-blind.



Oregon: grayish, yellow tinged with orange  
 svr<sup>poi</sup>: light yellow, neither gray nor orange  
 sp<sup>a</sup>: dark yellow-gray; abdomen, olive green tinge

The yellow of svr<sup>poi</sup> is pale, whitish, not the lemon yellow of the yellow mutant. The bristles are always dark but tend to be softer than usual. Newly hatched flies are yellower than older ones. Also, the pigmentation of the testis is yellow (dark in wild type). One of the characteristics of standard silver is the presence of a dark trident upon the thorax (which is highly modifiable in expression); this is absent in pointed and its alleles. However, in a line made up to contain white (w) together with pointed the trident appears. Pointed has, further, an inclination toward soft wings (see below) and toward spreading of the wings, but more so in other alleles to be described.

*Suppressor action.*—Pointed acts as a suppressor for speck, but not for vermillion (for sable see below). As a rule, sp/sp, svr<sup>poi</sup> in males shows no speck at all, but sp/sp, svr<sup>poi</sup>/svr<sup>poi</sup> females vary from no speck to an intermediate condition (which appears only as a very rare exception in males). Tests made with the available svr stocks never showed such a suppressor action. Pointed thus combines the action of silver and the near-by located suppressors; we shall return to this later in our discussion.

One remarkable detail ought to be added. If flies are made up with speck opposite a speck deficiency, and pointed in the first chromosome, they are pointed and speck. The suppressor action is inferior to the enhancing action (exaggeration) of the deficiency, a fact of considerable interest for phenogenetical deliberations.

As sp suppression by the specific suppressors said to be located near svr usually entails sable suppression also, a check for s<sup>a</sup> was made. Crossover flies containing in the same chromosome pointed and sable forked were less dark than sable, and the dark trident which is very typical for s<sup>a</sup> was about one-third lighter than in the s<sup>a</sup> brothers without poi. Pointed thus is also a part suppressor for sable. This, by the way, represents a rather complex phenogenetical situation: svr has a trident; svr<sup>poi</sup> not, but has it in the presence of w; but the sable trident is partly suppressed by svr<sup>poi</sup>. The speck suppression also has its modifiers. If the entire X chromosome of pointed to the right of this locus is replaced by crossing over—w was used as a marker,—sp is only half suppressed in the males.

*Localization.*—Compounds svr<sup>poi</sup>/svr are always pale but do not always show the pointed wing tip; however, as a whole they appear as expected if pointed is a silver allele. In heterozygotic condition svr<sup>poi</sup> sometimes shows a very slight dominance effect of the pointed feature, which must be due to modifiers. Genetic localization was not easy, because crossing over in the tip of the first chromosome is known to be unreliable. For example, in tests of the y-w region in one experiment there were only 3 crossover males among more than 800 males and all these three were yellow, not pointed. In another experiment with the same loci, 499 ♀ 417 ♂ noncrossover, 3 ♀ 3 ♂ y 3 ♀ 2 ♂ w, both not pointed, were obtained. Thus pointed did not show with one of the loci, but crossing over was reduced in this region, though not by much. But later the visible crossover combination with w was obtained, though never with y.<sup>a</sup> Another experiment with prune (pn 0.8) gave no crossovers among

<sup>a</sup> K. Brehme (Proc. Nat. Acad. Sci. Wash., 27 [1941], 254-261) states that the combination y with svr is lighter than either alone. We never succeeded in finding the combination with svr<sup>poi</sup>.

almost 1,300 flies; kurz (1.7) gave no crossovers with 700 flies; with *sc ec* only 3 scute not *ec* (and no *ec* not *sc*) males were found among nearly 1,300 flies, some of them pointed. Obviously, *y* does not permit pointed to express itself. This might be a consequence of the fact that both loci control a body color, thus *y* being epistatic over *svr<sup>poi</sup>*, including the pointed wing effect. Some of the other crossover recombinations were not reliable phenotypically. The experiments were later repeated with a selected *poi* stock which showed the character very clearly, and crossovers were found. Table 2 presents the results.

The first two tests give a locus between 0.6 and 0.7. The *fa* test has a value to the right of *fa*, but as *fa* is not easily classified the result is hardly reliable. The *ec* test puts *poi* to the left of *w*. Actually the *poi* crossover class appears much smaller than the reciprocal one, probably because of poor expressivity; sex-linked lethals in the *poi* chromosome which can be responsible will, however, be described later. If we calculate only on the basis of the plus class, the value becomes 0.1 for the

TABLE 2  
LOCALIZATION OF *svr<sup>poi</sup>*

	Number of flies	C.o.	Per cent	Locus of marker	Reciprocal c.o. classes	
					+	<i>poi</i> marker
C.o. <i>poi-pn<sup>a</sup></i> .....	1357	2	0.14	0 8	..	2
C.o. <i>poi-w</i> .....	852	7	0 81	1. 5	6	1
C.o. <i>poi-fa</i> .....	675	36	5 06	3 0	20	16
C.o. <i>poi-ec</i> .....	938	41	4.19	5. 5	27	14
					53	33

experiment with *w*, and zero with *ec*. Silver is located at 0.1. For a long time we had assumed that *poi* is a translocation of the *ll sp* loci into the first chromosome to the left of *white*, because an insertion of 2-3 bands was found here in the salivaries and the crossover values with *ec* could be interpreted in favor of this (also the *sp* suppression). But later tests showed the *sp* suppressor action to be independent of a duplication and in the nature of other suppressor actions in that region, and the compound actions showed that *poi* must be a silver allele. The decisive check is obtained by crossing pointed to L. V. Morgan's deficiency and duplication 101, which is deficient for the left end of X including silver. Silver × Df 101 was used as control. Actually, the heterozygous deficiency effect (without exaggeration [once a compound *svr*/Df showed exaggeration]; also in *svr*) was found—with decreased viability,—thus proving that pointed is covered by the deficiency and actually is a silver allele. But this is not the whole story, as will be shown later in an analysis of the X chromosomes of the silver group and in a discussion of the features of this region. The translocation which first was suspected is much farther to the right, beyond facet.

*An independent allele of pointed*—Independently of the present work we found an allele of pointed which closely resembles this, though there are a few differences. It was found in the offspring of heat-treated flies and had been isolated as a sex-linked recessive, indistinguishable from the present pointed wing except that the

pointed wing tip is usually better expressed in this mutant. The pedigree of this mutant *poi h* (*h* for heat) is:

$F_1$  ♀♂ Oregon, heat-shock shortly before pupation: 12 ♀♀, 9 ♂♂ +  
 $F_2$  =  $F_1$  Mass: 213 ♀♀, 233 ♂♂, 1 ♂ notched  
 $R F_3$  = Oregon ♀ × notched ♂  $F_2$ : all +, but 1 ♂ notched and *poi*  
 $F_4$  Mass  $F_4$  +: 400 ♀♂ +, 19 ♂♂ *poi*

In crosses they behaved as alleles, but they are in some respects, apart from the pointed phenotype, genetically different, as will be shown below. We shall call the latter mutant *svr<sup>poi h</sup>* (abbr.: *poi h*.)

*Expressivity.*—The expressivity of the phenotype of pointed is important for our later discussion of its origin. The pale body color and the suppression of speck is always clear, though in the presence of plexus the body color is not always distinctive. But the pointed wings are rather variable, except in selected stock, though less so than in silver. It turned out that our selected stock of *svr<sup>poi</sup>* and also of *svr<sup>poi h</sup>* contained definite dominant modifiers. (We have already seen that yellow [*y*] prevents the expression of pointed wings; but this might be called an epistatic action.) They first became clearly visible in translocation tests, i.e., *y*, *bw*, *e*, *ey* ♀ × (*y*, *bw*, *e*, *ey* × *poi*) ♂. In the  $F_1$  different results had already appeared. Sometimes all the sons of *y* × both pointed alleles were clearly pointed; sometimes the character appeared intermediate, with a varying number of soft and spread wings (which is an irregular feature of the pointed phenotype and especially of certain alleles; see below); sometimes the males might have passed for normal. The backcross behaved in the same way, and there also appeared a relation between the expressivity and the autosomal recombinations. The same features were observed for sons of pointed mothers and other fathers. On table 3 a series of such crosses was checked for the expressivity of pointed in three grades A, B, C (also for sex ratios; see below). The considerable variability becomes evident, which in this case must be based upon the presence of modifiers in the stock used for crossing. Actually, some of the series are rather consistent, e.g., a *sp* crosses: always full expression; *y w* crosses: medium; *bw sp ba* crosses: poor expression. The column "Remarks" shows that frequently the pointed  $F_1$  males are also soft or soft and folded.

There is, further, a considerable inclination toward spread wings, which characterizes also lanceolate (*ll*), i.e., a similar phenotype at a different locus. In one case  $\frac{1}{2}$  of the males were more or less blistered. Here the balloon locus was involved (actually a mutant *bran* had appeared); we shall return to this case. It is remarkable that one blistered female appeared in one of the Lausanne crosses, as we had found (see below in chapter on blistering) that the Lausanne stock of wild flies tends to show blistered wings.

We now compare these data with some for the allele *svr<sup>poi h</sup>*, which is not related to *px bl* and *svr<sup>poi</sup>*. Table 4 registers these data. We notice that as a rule the pointed character of the males is much better expressed than in the crosses with *svr<sup>poi</sup>*. Unclassifiable or actually not pointed males do not occur. (But this does not apply to  $F_2$ ; see below.) It was frequently noted that the character was best expressed in broods with a low sex ratio. Only rarely do soft males appear, but they are found, thus showing that this allele also has the same tendency, though a lower one, to produce soft wings. A very frequent occurrence is females and, more rarely, males with abnormal abdomen. In the C series there are a few in almost every brood.

TABLE 3

svr<sup>pol</sup> ♀ × N ♂

(Grades of pointed: A, type; B, intermediate; C, poor)

No.	Father	♀	♂	Grade of pointed ♂	Remarks	Sex ratio n ♀ : 1 ♂
5682 B	triple	74	36	A	.....	2 1
5705 B	X ple	75	39	B	Most ♂ soft folded.....	1 9
5710 B	ey	57	37	A	Most ♂ soft folded.....	1.5
6197	5 ple	121	47	A	1 ♂ spread.....	2 6
6289 B	Lausanne	18	10	A, B	.....	1.8
6290 B	Lausanne	127	68	A, B	1 ♀ blist.....	1 9
6291 B	Lausanne	114	43	A, B	.....	2.6
6320 B	px bw sp	96	31	A, B	.....	3.1
6322 B	a sp	24	2	A	.....	12.0
7824 B	a sp	111	92	A, 2 ♂ +	.....	1.2
7825 B	a sp	115	69	A	.....	1.7
2826 B	a sp	119	57	A 4 ♂ ?	.....	2.1
7827 B	a sp	86	54	A	.....	1.6
7828 B	a sp	115	84	A 2 ♂ ?	.....	1.4
7829 B	a sp	70	36	A	.....	1.9
7831 B	a sp	118	69	A	.....	1.7
3061 C	y w	52	57	B	Many spread.....	0.9
3062 C	y w	39	44	B	Many spread.....	0.9
3064 C	y w	74	62	B	Many spread.....	1.2
3070 C	y w	15	15	B	Many spread.....	1.0
3082 C	bw sp ba	48	17	C	.....	2.8
3083 C	bw sp ba	65	42	C	Some spread.....	1.5
3084 C	bw sp ba	59	46	C	.....	1.3
3085 C	bw sp ba	57	35	C	Half ♂ also soft and spread blistered	1.6
3086 C	bw sp ba	36	27	C	.....	1.3
3087 C	bw sp ba	93	46	B, C	Many ♂ soft.....	2.0
3088 C	bw sp ba	84	61	B, C	Many ♂ soft.....	1.4
3089 C	bw sp ba	45	31	B, C	Many ♂ soft.....	1.4
3090 C	bw sp ba	75	59	B, C	Many ♂ soft.....	1.3
3107 C	b j p	54	47	B	.....	1.1
3124 C	triple	66	57	B	.....	1.2
3125 C	triple	88	96	B	Most ♂ soft folded.....	0.9
3126 C	triple	135	84	A, B	.....	1.6
3128 C	triple	84	58	B	Most ♂ soft folded.....	1.4
3129 C	triple	84	52	A, B	.....	1.6
3131 C	triple	127	97	A, B	.....	1.3
3132 C	triple	125	77	A, B	.....	1.6
3147 C	y a c	136	71	A	.....	1.9
3148 C	y a c	102	72	A	.....	1.4
3149 C	y a c	80	71	A, B	.....	1.1
3163 C	ec ct g	34	19	B, C	.....	1.8
3164 C	ec ct g	34	28	B, C	.....	1.2
3165 C	ec ct g	45	33	B, C	.....	1.4
3166 C	ec ct g	46	33	A, B	.....	1.4
3167 C	ec ct g	14	25	A, B	.....	0.6
3171 C	ec ct g	76	52	A, B	.....	1.5
3172 C	ec ct g	21	14	A, B	.....	1.5
Sa.		3533	2302		.....	1.5
Avg.		75	49			

TABLE 4  
svr<sup>poi</sup> × N ♂

No.	Father	♀	♂	Grade of pointed ♂	Remarks	Sex ratio n ♀ : 1 ♂
5662 B	5 ple	62	25	A B	11 ♀ 2 ♂ abnormal abdomen (not inherited).....	2 5
5663 B	5 ple	55	12	A B	4 ♀ 1 ♂ abnormal abdomen.....	4 6
5699 B	X ple	95	69	A	.....	1 3
5701 B	triple	31	9	A	♂ soft.....	3 4
5702 B	triple	35	25	A B	.....	1 4
6424 B	X ple	113	52	A B	.....	2 2
6168 B	5 ple	96	11	A	.....	8 7
6169 B	5 ple	119	37	A	.....	3 2
6170 B	5 ple	41	5	A	.....	8 2
6171 B	5 ple	82	4	A	.....	20 5
6172 B	5 ple	125	43	A	.....	3 0
3071 C	y w	27	28	A B	.....	1 0
3072 C	y w	63	33	A	Pointed character more marked where fewer males.....	1 9
3073 C	y w	23	22	A B	In all the crosses series C frequently ♀ with abnormal abdomen.....	1 0
3075 C	y w	81	55	A	.....	1 5
3076 C	y w	41	27	A	.....	1 5
3077 C	y w	66	33	A	.....	2 0
3078 C	y w	51	25	A	.....	2 0
3079 C	y w	65	51	A B	.....	1 3
3080 C	y w	54	42	A B	.....	1 3
3091 C	bw sp ba	120	76	A B	.....	1 6
3092 C	bw sp ba	29	26	A B	.....	1 1
3093 C	bw sp ba	94	72	A B	.....	1 3
3094 C	bw sp ba	35	7	A B	.....	5 0
3095 C	bw sp ba	110	74	A B	.....	1 5
3097 C	bw sp ba	23	35	A B	.....	0 7
3098 C	bw sp ba	94	55	A B	.....	1 7
3099 C	bw sp ba	74	56	A B	1 ♂ spread blistered.....	1 3
3100 C	bw sp ba	14	5	A B	.....	2 8
3114 C	b j p	39	37	A B	.....	1 1
3115 C	b j p	38	14	A B	.....	2 7
3116 C	b j p	13	12	A	.....	1 1
3117 C	b j p	85	54	A B	.....	1 6
3118 C	b j p	28	10	A B	.....	2 8
3119 C	b j p	30	13	A B	.....	2 3
3121 C	b j p	75	65	A B	.....	1 1
3122 C	b j p	98	73	A B	.....	1 3
3133 C	triple	109	97	A B	.....	1 1
3135 C	triple	93	88	A B	Many ♂ soft folded.....	1 1
3136 C	triple	72	45	A	.....	1 6
3137 C	triple	50	40	A B	.....	1 3
3138 C	triple	111	107	A B	Many ♂ soft folded.....	1 0
3139 C	triple	91	56	A	.....	1 6
3140 C	triple	91	71	?	.....	1 3
3141 C	triple	89	44	A	.....	2 0
3142 C	triple	140	124	A B	.....	1 1

TABLE 4—(Continued)

No.	Father	♀	♂	Grade of pointed ♂	Remarks	Sex ratio n ♀ : 1 ♂
3153 C	y a c	109	68	A B	.....	1.6
3154 C	y a c	131	76	A B	.....	1.7
3155 C	y a c	112	83	A B	.....	1.4
3156 C	y a c	116	98	A B	.....	1.2
3157 C	y a c	111	60	A B	.....	1.8
3158 C	y a c	88	80	A B	.....	1.1
3159 C	y a c	25	29	A B	.....	0.9
3160 C	y a c	68	85	A B	.....	0.8
3161 C	y a c	93	54	A B	.....	1.7
3162 C	y a c	96	56	A B	.....	1.7
3173 C	ec ct g	109	93	B	.....	1.2
3174 C	ec ct g	121	72	A	.....	1.7
3175 C	ec ct g	92	74	A	.....	1.2
3178 C	ec ct g	101	74	A B	.....	1.4
3179 C	ec ct g	77	67	B	.....	1.1
3180 C	ec ct g	101	90	B	.....	1.1
Sa.		4700	3122		.....	1.5
Avg.		76	51			

Many have been tested, and the character was never inherited. But we shall later see that a genetic type of abnormal abdomen is obtained from poi involving the bb locus.

The modification of expressivity just recorded is of minor significance. But there is another, more important, one which became visible in  $F_2$  and backcrosses, in which half the males or females and males ought to be pointed. Table 5 contains a series of reciprocal  $F_2$  for both alleles with Oregon.

Looking at the summarized results, a few regularities become visible: (1) Never is the ratio + : poi the expected 1:1 in either set (checking only the wings, not the pale color; see below : viability). (2) Where both sexes contain pointed flies the ratio for females is much higher than for males. In both these cases the sex ratio is normal, the ratio of + ♀ : + ♂ is also normal, and therefore about the same number of genetically pointed individuals do not exhibit the character in both sexes. In the  $svr^{poi}h$  crosses, however, there are still more males which do not show the character. In the average here, a little more than  $\frac{1}{2}$  of the females homozygous for poi, but about  $\frac{2}{3}$  of the males, do not show the character. This suggests that a recombination with two or three dominant enhancers introduced from the poi stock may be responsible for the lack of expressivity of pointed. (3) The reciprocal crosses in which the poi-containing first chromosome was introduced by a poi father are different. First, the sex ratio is a very high one, about  $\frac{1}{3}$  of the males missing (see below). The proportion of males exhibiting pointed is only about  $\frac{1}{3}$  of all the males. The ratio of + ♀ : + ♂ is 1.7:1. If we assume that just as many poi males are phenotypically plus as in the reciprocal cross, we can subtract these from the plus males. The ratio was 17:10 in the opposite cross, which means that in the present cross about 170 males in the plus class are supposed to be genetically pointed. Subtracting these, the ratio 2 ♀ : 1 ♂ + is approximately realized. From this it follows that between  $\frac{1}{2}$  and  $\frac{5}{8}$  of the poi males are lethal. In other words, the X chromosome, if introduced

by a poi male, contains something which is lethal in connection with about 5 out of 8 autosomal recombinations; this problem will be studied below. (4) The individual ratios are arranged so as to give information on whether different grandparents of the same cross may have a specific influence (the number of  $F_1$  means always one

TABLE 5  
RECIPROCAL  $F_2$  OF  $svr^{poi}$  AND  $svr^{poi h}$  WITH WILD TYPE 7145/73 Br.

No.	Cross	+		poi		Soft folded		Ratio $\varphi:1\sigma$	Ratio + : poi		Ratio + $\varphi$ : + $\sigma$
		$\varphi$	$\sigma$	$\varphi$	$\sigma$	$\varphi$	$\sigma$		$\varphi$	$\sigma$	
7145	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7079 <sup>2</sup> .....	69	59	17	..	..	16	1.1	4.0	3 7	1.2
7146	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7079 <sup>2</sup> .....	82	87	25	4	..	9	1.1	3.3	6.7	0.9
7147	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7079 <sup>2</sup> .....	56	47	9	..	22	32	1.1	1.8	1.5	1.2
7148	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7079 <sup>2</sup> .....	47	62	31	13	..	..	1.0	1.5	4 8	0.8
7149	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7081 <sup>2</sup> .....	59	63	13	2	13	17	1.0	2.3	3.3	0.9
7150	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7081 <sup>2</sup> .....	71	73	22	5	1	2	1 2	3.1	10 4	1.0
7151	( $svr^{poi} \times Ore$ ) <sup>2</sup> 7081 <sup>2</sup> .....	87	64	15	6	9	25	1.2	3.6	2 1	1.4
7153	( $Ore \times svr^{poi}$ ) <sup>2</sup> 7082 <sup>2</sup> .....	103	91	..	7	..	..	1.1	..	13.0	1.1
7154	( $Ore \times svr^{poi}$ ) <sup>2</sup> 7082 <sup>2</sup> .....	116	75	..	4	..	11	1.3	...	4 0	1 6
7156	( $Ore \times svr^{poi}$ ) <sup>2</sup> 7083 <sup>2</sup> .....	120	78	..	8	..	3	1.3	...	7.0	1.5
7157	( $Ore \times svr^{poi}$ ) <sup>2</sup> 7084 <sup>2</sup> .....	77	52	..	..	..	..	1.5	...	...	1.5
7158	( $Ore \times svr^{poi}$ ) <sup>2</sup> 7084 <sup>2</sup> .....	82	34	..	..	..	..	2.4	...	...	2.4
7159	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7088 <sup>2</sup> .....	88	81	..	13	..	..	0.9	...	6.0	1.1
7160	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7088 <sup>2</sup> .....	77	92	14	5	..	..	0.9	5.5	18.0	0.9
7161	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7088 <sup>2</sup> .....	85	88	32	9	..	..	1.2	2.7	9.8	1.0
7162	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7088 <sup>2</sup> .....	69	78	30	8	..	..	1.2	2.3	9.7	0.9
7163	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7088 <sup>2</sup> .....	97	109	22	6	..	..	1.0	4 5	18.0	0.9
7164	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7089 <sup>2</sup> .....	116	122	12	..	6	21	0.9	6.4	0.0	1.0
7165	( $svr^{poi h} \times Ore$ ) <sup>2</sup> 7089 <sup>2</sup> .....	106	122	17	5	..	5	0.9	6.2	12.2	0.9
7167	( $Ore \times svr^{poi h}$ ) <sup>2</sup> 7090 <sup>2</sup> .....	161	87	..	14	..	..	1.6	...	6.2	1.9
7168	( $Ore \times svr^{poi h}$ ) <sup>2</sup> 7090 <sup>2</sup> .....	135	82	..	13	..	..	1.4	...	6.3	1.6
7169	( $Ore \times svr^{poi h}$ ) <sup>2</sup> 7091 <sup>2</sup> .....	138	67	..	11	..	..	1.8	...	6.1	2.1
7170	( $Ore \times svr^{poi h}$ ) <sup>2</sup> 7091 <sup>2</sup> .....	124	52	..	17	..	..	1.6	...	3.1	2.4
7171	( $Ore \times svr^{poi h}$ ) <sup>2</sup> 7092 <sup>2</sup> .....	141	89	..	7	..	..	1.5	...	12.7	1.6
7172	( $Ore \times svr^{poi h}$ ) <sup>2</sup> 7092 <sup>2</sup> .....	119	79	..	9	..	..	1.4	...	8 8	1.5
	All ( $svr^{poi} \times Ore$ ) <sup>2</sup> .....	471	455	132	30	45	101	1.1	2.7	3.5	1.0
	All reciprocal.....	408	330	...	19	..	14	1.4	...	10.0	1.5
	All ( $svr^{poi h} \times Ore$ ) <sup>2</sup> .....	638	692	127	46	6	26	1.0	4.8	9.6	0.9
	All reciprocal.....	818	456	...	71	..	..	1.6	...	6 4	1.8
	All grandmother poi.....	1109	1147	259	76	51	127	1.1	3.6	5.7	1.0
	All grandfather poi.....	1316	786	...	90	..	14	1.5	...	7.6	1.7

pair mating, and different numbers, different pairs). There are two sister  $F_2$  7157, 58 without pointed males; but this is not significant in this group. There are twice (7164-65, 7171-72) sister broods with higher poi ratios than the others in the group; but this is not necessarily significant.

The best method for checking upon the supposed enhancers is to cross pointed to  $\underline{y}$ , bw, e, ey, and  $F_1$  males back to  $\underline{y}$ , bw, e, ey  $\varphi$ , the standard translocation test. (Patterson test). All sons from these crosses have the poi X chromosome with different autosomal recombinations: either heterozygous for one or the other of the poi autosomes, or homozygous for foreign chromosomes. If  $F_1$  had all pointed males

(which is not always the case; see above), all the dominant enhancers are present in the heterozygote and the backcross must give information.

We have mentioned already that, in  $F_1 \underline{y} \times$  both alleles, all males are sometimes clearly pointed and sometimes not. As an example table 6 may serve. Many other crosses gave the same results, namely, sometimes the  $F_1$  males are clearly pointed, all of them or some of them; sometimes pointed is not clear at all, and sometimes it cannot be distinguished. (This means only the wing shape, not the pale color.) But whenever these males were backcrossed to pointed females they turned out to be genetically pointed, siring only pointed offspring. The other features, such as appearance of soft folded and spread males and the varying sex ratios, are the same as in ordinary crosses, and the males which did not exhibit pointed wings were pale.

In a large backcross series with  $\underline{y}$ , bw, e, ey, involving 17 with  $svr^{poi}$  and 6 with

TABLE 6  
 $F_1 \underline{y} \times$  pointed (Patt =  $\underline{y}$ , bw, e, ey)

No.	Mother	Father	♀	♂ point	♂ ± point	♂ ± no	Remarks
5755	Patt	$svr^{poi}$	48	21	21	..	12 ♂ soft folded 4 ♂ spread
5762	Patt	$svr^{poi}$	36	15	..	..	
5853	$\underline{y}$	$svr^{poi}$	76	60	..	19	
6193	Patt	$svr^{poi}$	28	..	..	12	
6223	Patt	$svr^{poi h}$	22	..	8	..	
6224	Patt	$svr^{poi h}$	15	..	7	..	
6225	Patt	$svr^{poi h}$	23	..	14	..	
Sa.	....	.....	248	..	193	..	

$svr^{poi h}$  and about 800 ♂, it was found consistently that all plus males were typically pointed, also all eyeless males. All brown males with or without eyeless were about intermediate; all ebony males, with or without eyeless, were very little pointed; and males both ebony and brown, with or without eyeless, were to all purposes normal. This shows that "dominant" modifiers for wing shape are present in the second and third chromosomes of pointed, and the more powerful ones in the third. It may be of interest in this connection that the pointed wing develops from a wing completely normal at the time of pupation (Goldschmidt, 1937a), the histological events being unknown. One should expect that a character fixed so late in development would be likely to respond easily to modifying action, genetic and otherwise.

*Heterozygous effects.*—In the  $F_1$  crosses with marked stocks another feature was found regularly. In crosses of  $svr^{poi}$  with stocks containing ebony, alone or in combination with other markers, a dominance effect was observed which first suggested a deficiency but did not turn out to be one. The effect is rather irregular, sometimes hardly observable, sometimes found in all females and males, which look like the ebony allele sooty. Sometimes even a part of the flies, especially males, look like a weak ebony with dark wings in addition to the body color. Table 7 contains some of the notes. The table shows clearly the dominance shifting effect of  $svr^{poi}$  for ebony. As the effect appears in crosses in both directions and therefore also in males without the  $svr$  mutant in the X chromosome (and also in backcrosses), it is autosomal in nature and may be due actually to the e locus of  $svr^{poi}$ . The allele  $svr^{poi h}$ , however, never produces this effect, which, then, is clearly derived from the px bl stock but



must be connected with the mutation to  $svr^{poi}$ , as it is not found in the px bl line. Rudimentary, which was produced together with pointed, shows the same effect! The difficulty of classification and the variability of the effect did not permit a direct localization (see below: third chromosome tests). It has already been mentioned that silver tends to show a trident on the thorax, shining through the pale cuticle. The pointed alleles never exhibit a trident. The e effect has nothing to do with this feature of the svr locus, as neither the heterozygote with black, nor compound with

TABLE 7

## CROSSES INVOLVING pointed AND ebony

(Color grades: I, weak trident and normal; II, trident; III, sooty; IV, low ebony)

No.	Cross	Color	Remarks
6173 B	$svr^{poi} \times \text{triple}$ ...	Part ♀ ♂ III .....	Some ♀ ♂ ski wings, with and without III. 5 ♀ blist Many ski wings
6174 B	reciprocal.....	All II, III .....	
6448 B	$\text{triple} \times svr^{poi h}$ ...	All normal.....	
6450 B	$\text{triple} \times svr^{poi h}$ ...	All normal.....	
6501 B	$\text{triple} \times svr^{poi h}$ ...	All normal.....	
6512 B	$\text{triple} \times svr^{poi h}$ ...	All normal.....	
6478-86 B	$svr^{poi h} \times \text{triple}$ ...	7 broods normal.....	
6487 B	$svr^{poi} \times \text{triple}$ ...	II .....	
6488 B	$svr^{poi} \times \text{triple}$ ...	II .....	
6489 B	$svr^{poi} \times \text{triple}$ ...	II .....	
6490 B	$svr^{poi} \times \text{triple}$ ...	II, III.....	
6491 B	$svr^{poi} \times \text{triple}$ ...	II .....	
6493 B	$svr^{poi} \times \text{triple}$ ...	II .....	
6494 B	$\text{triple} \times svr^{poi}$ ...	II-IV (about $\frac{1}{2}$ ♀ ♂ IV)	
6495-98 B	$\text{triple} \times svr^{poi}$ ...	3 broods all III and IV..	
6500 B	$\text{triple} \times svr^{poi}$ ...	II .....	
6426-44 B	$\text{triple} \times (\text{triple} \times svr^{poi}) \text{ ♂ III-IV}$	All not triple II-III....	

silver, shows the trident. We anticipate that we shall find evidences for a rearrangement in the e region so that the heterozygous effect might be actually a position effect.

The same mutant produces other dominance-shifting effects. In crosses with second-chromosome markers the locus arc is frequently semidominant. For example, in no. 6280 quintuple  $\times svr^{poi}$ , out of 42 ♀ 46 ♂, there were 14 ♀ 7 ♂ more or less arc, but none perfect. Again, deficiency tests failed and the irregularity was the same as for ebony. The number of individuals showing the dominance effect was always small. Furthermore, this effect was not typical for  $svr^{poi}$ , but was also found in the allele  $svr^{poi h}$ . It was clearly related to the action of the svr locus, as only pointed females and males showed the effect, not the heterozygous females. We remember at once that bran is an arc allele and that  $svr^{poi}$  tends to produce bran as a mutant. Again the possibility of a position effect with a disturbance at or near the arc locus is suspected (see below).

There is a third similar effect, more interesting than the two just described. In a series of crosses with both silver alleles and the first-chromosome locus achete, in a majority of broods about one-half of the females were achete, though the character

was again less clearly expressed than in the pure achete stock, and more variable. Many tests were made to clear up this effect, as the silver locus is located not so far away from yellow and achete, and as it was frequently observed that  $svr^{poi}$  individuals, especially males, tended to have a light yellowish (lemon) tinge, besides being pale. (The mutant achete, by the way, had originated in all individuals of a

TABLE 8  
BRISTLES IN  $svr$  CROSSES

No.	Cross	♀	♂	Bristles
3696 C	$poi^{px\ bl} \times Inv(1)y^{px\ bl} \dots$	111	95	d.c.p. 12 ♀
3697 C	$poi^{px\ bl} \times Inv(1)y^{px\ bl} \dots$	109	50	50 ♀ 26 ♂ bb.
3698 C	$poi^{px\ bl} \times Inv(1)y^{px\ bl} \dots$	30	28	+
3710 C	$poi \times Inv(1)y^{px\ bl} \dots$	60	33	6 ♀ d.c.p.
3711 C	$poi \times Inv(1)y^{px\ bl} \dots$	73	64	22 ♀ d.c.p.
3713 C	$poi^{px\ bl} \times y\ ac \dots$	97	?	Little bb.
3714 C	$poi^{px\ bl} \times y\ ac \dots$	100	53	+
3715 C	$poi^{px\ bl} \times y\ ac \dots$	128	90	1 ♀ d.c.p.
3716 C	$poi^{px\ bl} \times y\ ac \dots$	112	67	+
3717 C	$poi^{px\ bl} \times y\ ac \dots$	83	52	7 ♀ d.c.p., 1 also d.c.a.
3873 C	$poi \times Ore \dots$	97	28	All ♀ much bb, $\frac{1}{2}$ ♂ bb.
3874 C	$poi \times Ore \dots$	100	95	All ♀ ♂ bb.
3875 C	$poi \times Ore \dots$	135	60	$\frac{1}{2}$ ♀, few ♂ d.c.p.
3876 C	$poi \times Ore \dots$	116	79	Almost all bb.
3877 C	$poi \times Ore \dots$	120	77	Almost all ♀, about $\frac{1}{2}$ ♂ bb.
3878 C	$poi^{px\ bl} \times Ore \dots$	138	75	15 ♀ d.c.p.
3879 C	$poi^{px\ bl} \times Ore \dots$	154	82	All ♀, few ♂ bb.
3880 C	$poi^{px\ bl} \times Ore \dots$	91	54	Almost all bb.
3881 C	$poi^{px\ bl} \times Ore \dots$	138	69	Almost all ♀, few ♂ bb.
3881 C	$poi^{px\ bl} \times Ore \dots$	100	96	$\frac{1}{2}$ ♀, few ♂ bb.
3812 C	3696 ♀ d.c.p. ....	78	87	All +, many similar F <sub>2</sub> bb or only few d.c.p.
3843 C	3697 $\times Inv(1)y^{px\ bl} \dots$	131	145	♀ y 48 + 31 bb ♂ poi 50 + 10 d.c.p. ♀ not y 30 + 22 d.c.p. ♂ y 55 + 33 bb.
3844 C	3697 $\times Inv(1)y^{px\ bl} \dots$	...	...	Only few ♀ y d.c.p. others and ♂ +
3861 C	3710 ♀ + ....	...	...	Most ♀ few ♂ all classes bb.
3862 C	3710 ♀ d.c.p. ....	...	...	$\frac{1}{2}$ ♀ poi or not poi d.c.p. ♂ +
3863 C	3711 ♀ + ....	...	...	$\frac{1}{2}$ ♀ ♂ poi or not d.c.p.
3864 C	3711 ♀ d.c.p. ....	...	...	All ♀, $\frac{1}{2}$ ♂ both classes bb.

yellow brood.) A repetition of the cross yielded 12 broods without an achete effect and 2 with a slight effect. This led to a closer investigation. Repeated checks of cultures of both  $svr$  alleles always showed normal bristles, even comparatively long ones. But in the majority of crosses, F<sub>1</sub> and later generations, abnormalities are found, whatever the cross. The bristle effect turned out to be completely independent of the  $svr$  locus, and also of achete, and to be a purely autosomal effect connected with the  $poi$  mutation, as it is found in both alleles. (We shall see below that  $svr^{poi}$  frequently contains a bb allele without visible effects except in compounds. This bb allele is not involved in the present discussion). Table 8 contains some pertinent data for F<sub>1</sub>. The crosses were made with: a small Inversion, to be described later as  $I(1)y^{px\ bl}$ , with one break in the y-ac region; with y ac standard stock; and with Oregon wild males. The results show a considerable variation:

normal bristles in  $F_1$ ; only the posterior dorsocentrals (d.c.p.) affected, with one or both missing in females or in both sexes, and in a few or many individuals; occasionally, also, the anterior dorsocentrals affected (d.c.a.); some bristles shortened (phenotype as in *bb*), or more or all shortened to a condition resembling spineless. In this latter type the scutellars lead. This extreme effect may be found in all females and males, mostly in females or in part of them in many variations, as the table shows. There is no relation to the *y* or *ac* locus, as the Oregon crosses show even more of the effect than those with mutants or breaks in the *y-ac* region.

TABLE 9  
FERTILITY EXPERIMENT

Cross	No. fertile	No. sterile	Fertility (per cent)
Ore-R ♀ × Ore R ♂	111	0	100.0
px bl ♀ × Ore R ♂	99	5	95.2
svr <sup>poi</sup> ♀ × Ore R ♂	110	6	94.8
slender ♀ × Ore R ♂	97	8	92.3
svr <sup>poi h</sup> ♀ × Ore R ♂	95	11	89.7
T px ♀ × Ore R ♂	93	7	93.0
poi bl ♀ × Ore R ♂	97	3	97.0
px from poi ♀ × Ore R ♂	98	4	96.07
soft bl ♀ × Ore R ♂	94	7	93.06
Ore R ♀ × px bl ♂	101	7	93.5
Ore R ♀ × svr <sup>poi</sup> ♂	98	2	98.0
Ore R ♀ × slender ♂	98	2	98.0
Ore R ♀ × svr <sup>poi h</sup> ♂	94	6	94.0
Ore R ♀ × T px ♂	99	1	99.0
Ore R ♀ × poi bl ♂	92	11	89.3
Ore R ♀ × px from poi ♂	97	8	92.3
Ore R ♀ × soft bl ♂	80	23	77.6

There is, further, no relation to the sex ratio, and though the males tend to show less of the effect, it is sometimes present in all males with the *poi* X chromosome. The lower part of the table contains some checks upon  $F_2$ . These show a corresponding irregularity. The X chromosome obviously does not influence the result, which must be based upon some autosomal condition acting and segregating in an irregular way. Actually, the absence of the *poi* X chromosomes seems to enhance the bristle effect, as, at least in some cases, the homozygous *y* Inversion flies were more like *bb*—which, however, may be a specific effect of this inversion (see below).  $F_3$  from both *poi* and *y*  $F_2$  flies, however, were again irregular; for example:

4494 = 3861<sup>2</sup> *poi* and *bb*-like: only 2 d.c.p. ♀ among 60 *poi*.

4495 = 3861<sup>2</sup> *poi* +: the same (all *poi*)

4496 = 3861<sup>2</sup> ♀ *poi* *bb*-like, ♂ *poi*; all ♀ (146) no, ♂ (104) d.c.p. (all *poi*)

4497 = 3861<sup>2</sup> ♂ ♂ *y* *bb*-like; ½ ♀ ♂ *y* *bb*-like, ½ +

4500 = 3861<sup>2</sup> ♀ ♂ *y* *bb*, ♂ *y*: ¼ ♀, few ♂ d.c.p.

Further trials to elucidate this situation were completely unsuccessful. When repeated about one and one-half years afterward, the effect did not reappear. Obviously the underlying condition had been selected out of the stock. Probably one of the deficiencies found in the salivaries was responsible.

**Viability and fertility.**—The viability is lower than in normal flies, the average number of flies in a single-pair bottle, *ceteris paribus*, being about one-half the number in controls, which, however, as we shall see, may be caused by the presence of lethal classes. Also the fertility is a little lowered, as table 9 shows, more so in the females. This table, compiled for some of the mutants and their recombinations with mutants at the px, svr, a loci (derived from px bl), is based upon the following experiment: 1 ♀ 2 ♂ of the respective combinations were left in a bottle at 25° C. On the sixth day, bottles were discarded in which there was not at least the female and one male alive. The remaining bottles were reexamined on the tenth day and those

TABLE 10  
PHENOTYPES (No. 5426 ff.)

No.	Compound	Phenotype
1	Px <sup>1</sup> /ll sp. ....	Exagg. sp; medium plexation; web and not in I (see terminology in section on px bl)
2	Px <sup>2</sup> /ll sp. ....	No sp; low plexation; only few extra veins and branch of cross vein
3	Px <sup>1</sup> /bs. ....	♀ Web at cross vein, all blistered; ♂ web; extra venation in II
4	Px <sup>2</sup> /bs. ....	♀ Web at cross vein mostly blistered, ♂ antler-web
5	Px <sup>1</sup> /ba. ....	Web at cross vein, periclinial vein, net in II, ♀ much blistered, many ♂ blistered
6	Px <sup>2</sup> /ba. ....	The same, but no ♂ blistered and less net
7	Px <sup>1</sup> /+ ♀ svr <sup>poi</sup> /+, ♂ svr <sup>poi</sup> ....	Most ♀ ♂ low plexus more or less like Px <sup>1</sup> /+ ! some normal; ♂ poi pale
8	Px <sup>2</sup> /+ ♀ svr <sup>poi</sup> /+, ♂ svr <sup>poi</sup> ....	The same but less plexation; more or less like Px <sup>2</sup> /+
9	bs/+ ♀ svr <sup>poi</sup> /+, ♂ svr <sup>poi</sup> ....	Normal like bs/+ with a little dominance effect in ♀ (EV)
10	ba/+ ♀ svr <sup>poi</sup> /+, ♂ svr <sup>poi</sup> ....	The same

without larvae were called sterile. The sex ratio was about normal in both lines, e.g., in one count 385 ♀ 339 ♂ = 1.1 : 1 for svr<sup>poi</sup>, the greatest individual deviation in favor of females being 77 : 55. For svr<sup>poi</sup> <sup>h</sup> the count was 496 ♀ 441 ♂ = 1.1 : 1, the greatest deviation in favor of females being 55 ♀ : 36 ♂.

**Pointed and lanceolate (ll).**—Pointed wings look exactly like lanceolate wings (ll, 2d chr.), though lanceolate is not pale and has no suppressor action. A combination of a higher allele of pointed with a bran allele, called slender, looks exactly like ll<sup>2</sup>. Also both have a tendency to spread their wings. This might be just a phenotypical resemblance. At the time when I thought that pointed is a speck duplication (because there is a small translocation near facet in the pointed stock) many experiments were performed to check upon the relation of ll and poi. F<sub>1</sub> between the two is completely normal, but in F<sub>2</sub> and backcrosses strange ratios appeared, based upon a lethal combination (see below). But when ll could be recombined with pointed it was typical (while sp, in the combination ll sp/ll sp; svr<sup>poi</sup> was suppressed as always in the presence of poi. Lanceolate, then, is epistatic in this combination.

**Pointed and blistered, and balloon.**—The origin of pointed from px bl involved the disappearance of blistered contained in px bl. On the other hand, pointed itself produces blistering if combined with the second-chromosome arc allele bran. There-

fore a check is needed to ascertain whether pointed shows any special interaction with blistered (bs) or the near-by and similar balloon (ba), or whether it contains a bs allele without visible effect except in the presence of a mutant at the arc locus.

No trace of the extra veins characteristic of these stocks (bs, ba) was ever found in pointed. A simple test such as the one performed with ll sp was not promising, on account of the phenotypic difficulties offered by the mutants bs and ba and their heterozygotes. (See discussion of this subject in the section on px bl.) These difficulties could be evaded by using bs and ba in the exaggerated condition shown in the presence of the Plexate deficiencies. (For a detailed description of all these phenotypes see the section on px bl.) The procedure was the following: the Plexates—deficiencies for bs, ba (sp)—were crossed to both pointed alleles, ll, bs, and

TABLE 11  
EXPECTATION FROM (poi × bs) × (poi × Px<sup>1</sup>)

	2d chr.	1st chr.	Phenotype without interaction poi-bs
1/8	bs/+	♀ poi/poi	Like no. 9, table 10
1/8	bs/+	♀ poi/+	Like no. 9, table 10
1/8	bs/Px <sup>1</sup>	♀ poi/poi	Like no. 3, table 10
1/8	bs/Px <sup>1</sup>	♀ poi/+	Like no. 3, table 10
1/8	Px <sup>1</sup> /+	♀ poi/poi	Like no. 7, table 10
1/8	Px <sup>1</sup> /+	♀ poi/+	Like no. 7, table 10
1/8	+/+	♀ poi/poi	Normal
1/8	+/+	♀ poi/+	Normal

ba; and both pointed alleles were crossed also to bs, ba to check again upon the phenotypes. (Px<sup>1</sup> includes sp, Px<sup>2</sup> does not.) Table 10 describes these phenotypes, which are classifiable without any difficulty.

Now F<sub>1</sub> svr<sup>poi</sup> or svr<sup>poi h</sup> × bs was crossed with F<sub>1</sub> svr<sup>poi</sup> or svr<sup>poi h</sup> × Px<sup>1</sup>; the expectations from the combination (poi × bs) × (poi × Px<sup>1</sup>) are shown in table 11. The decisive group is the one with bs/Px<sup>1</sup>; which is expected to contain (one-fourth of the offspring) flies with much plexation with web and blisters, a type absent in all other combinations, which are less conspicuous and near normal. Also, the females of these two groups would be one-half pale, that is, homozygous poi, and one-half heterozygous.

The actual result for svr<sup>poi</sup> was (nos. 5505 and 5506):

110 ♀ 116 ♂ normal or almost normal types

19 ♀ 20 ♂ Px<sup>1</sup>/bs (blistered) type

18 ♀ 10 ♂ pointed, more plexus than first groups, no web, antler, or blisters.

This shows that homozygous pointed weakens the exaggeration effect of Px/bs.

The same test made for svr<sup>poi h</sup> (not derived from px bl) gave clearly 1/4 of the Px<sup>1</sup>/bs type and no interaction of the two loci; F<sub>3</sub> tests confirmed the result. Parallel crosses with Px<sup>2</sup> gave the same results as those with Px<sup>1</sup>. Finally, also the corresponding combination with the compound svr<sup>poi</sup>/svr<sup>poi h</sup> was built up. Actually (no. 5510), all individuals bs/Px<sup>1</sup> with the compound were blistered, etc., which confirms the difference found between the two pointed with a dominant action of

A similar test for balloon was made simpler, because balloon was used with near-by speck; as  $Px^1$  is a deficiency also for sp, all  $Px^1/sp$  ba individuals show speck. The very extreme plexate and blistered type of this compound is described in table 10, no. 5. The cross  $(poi \times Px^1) \times sp$  ba contains the answer (no. 5511):

not sp (i.e., not $Px^1/sp$ ba)	38 ♀ 42 ♂ ( $\frac{1}{2}$ ♂ poi)
sp (i.e., $Px^1/sp$ ba)	40 ♀ extremely px and blist. like $Px^1/ba$
	18 ♂ not poi, the same
	13 ♂ poi, no periclinial or parallel veins, no or little antler or net

that is,  $svr^{poi}$  has the same action on balloon exaggeration as on bs. A remarkable fact is that here the pointed males show speck, which is otherwise suppressed by the pointed locus. The exaggeration effect of the deficiency prevents this phenotypic suppression.

Thus we see that the  $svr^{poi}$ , not containing bs, from which it is derived, has a developmental interaction of a suppressor type both with bs and ba, located near each other just as was the case with the near-by locus sp.  $Svr^{poi^h}$  not derived from bs has no interaction with bs, ba. I have no explanation to offer for this interrelation which does not appear fortuitous, as there is no experiment in favor of a bs duplication in the poi chromosome. We shall meet later with a small insertion into the first chromosome of poi and poi h near facet which could contain the bs and ba loci, though the origin is unknown. But actually two fewer bands are present in poi than in poi h, which makes this translocation not available for an explanation of the facts just noted.

*Sex ratios and lethal classes.*—It has been noted in the proper columns of tables already presented that  $F_1$  and  $F_2$  from pointed crosses showed abnormal sex ratios, which might mean a differential viability of flies with the pointed X chromosome or genetic lethal conditions. The analysis revealed a very complicated situation which somehow must be connected with the production of the mutant. It will simplify the description if we first assemble the main results and derive a hypothetical interpretation before we present the data. The following apparently contradictory facts are decisive:

- Within the two pointed lines the sex ratio was always found normal.
- The same is true for reciprocal crosses between  $svr^{poi}$  and  $svr^{poi^h}$ , though the variation is larger than in the pure broods.
- The number of flies in the offspring of a pointed pair is about  $\frac{1}{2}$  of that in the controls.
- $F_1$  from wild type or marker ♀ and pointed ♂ have a normal sex ratio.
- $F_1$  from an attached X ♀ with poi ♂ has a normal sex ratio.
- The following  $F_1$ ,  $F_2$ , and  $RF_2$  contain, as a rule, male lethal classes in different ratios:  $poi \times N$  ♂;  $(poi \times N) \times N$ ,  $(N \times poi) \times N$ ,  $(N \times poi)^2$ .
- The backcrosses  $(poi \times N) \times poi$ ,  $(N \times poi) \times poi$ ,  $poi \times (N \times poi)$ ,  $poi \times (poi \times N)$  have, as a rule, a normal sex ratio.
- The combinations with abnormal sex ratios seem to contain different ratios, both in  $F_2$  and  $RF_2$ .
- The standard translocation text  $\underline{y}$ , bw, e ey  $\times$  (ditto  $\times$  poi) is negative.
- A test with backcrossing  $(poi \times bw \text{ e ey}) \times bw \text{ e ey}$  shows poi ♂ missing in definite classes and ratios.

1) Backcrosses with marked second or third chromosomes reveal lethal combinations of poi with definite regions of these chromosomes.

These data exclude ordinary lethal loci in the pointed X chromosome. They exclude ordinary reciprocal translocations with lethal duplicated or deficient classes; they require a difference between the pointed X directly derived from a male ( $N \times poi$ ,  $\underline{y} \times poi$ ) and such an X derived from a pointed female or a female heterozygous for pointed, whether the poi X is grandmaternal or grandpaternal. Thus they require nonreciprocal translocation from the poi X chromosome into autosomes, which produce male lethal deficiencies in the X of males and, in the proper crosses, in both sexes (which restores the normal sex ratio). Finally, the absence of lethal

TABLE 12  
FREQUENCY OF BROODS

Cross	♀ : 1 ♂															
	0	6	0	8	-1	0	-1	2	-1	4	-1	6	-1	8	-2	0
$svr^{poi} \times N$	1	3	3	8	11	7	6	3	..	..	2	1	1	..	1	
$svr^{poi h} \times N$	1	2	13	11	6	10	2	3	2	2	1	1	1	2	5*	
$svr^{poi h} \times svr^{poi}$	..	2	1	1	2	1	..	..	..	..	..	..	..	..	..	
$svr^{poi} \times svr^{poi h}$	1	1	3	2	2	..	..	..	..	..	..	..	..	..	..	

Cross	N	Mean sex ratios	Mean number in brood
$svr^{poi} \times N$	5835	1.5	124
$svr^{poi h} \times N$	7822	1.5	126
$svr^{poi h} \times svr^{poi}$	952	1.3	136
$svr^{poi} \times svr^{poi h}$	985	1.2	109

\* 309 ♀ 39 ♂.

classes in translocation tests with unbroken paternal poi chromosomes suggests the presence there of the translocated sections of the X chromosome from pointed as duplication, and this at a considerable distance from the deficiency to permit frequent crossing over. Thus the working hypothesis is: The X chromosome of pointed contains, in addition to the  $svr^{poi}$  mutant locus, (1) two nonreciprocal translocations from the X into the second and third chromosomes, respectively, (2) a simultaneous transposition of the same deficient loci into the regions to the right of the X chromosome, (3) variation and heterozygosity with respect to all these features within the stocks, and (4) parallel structure in both poi mutants.

We report now on the individual points ( $a-k$ , above).

a) Normal sex ratios within the pointed lines have been found again, to mention only the last check made for  $svr^{poi}$ , namely, 986 ♀ 895 ♂ = 1.1:1.

b) Tables 12-14 show the ratios and the frequency of broods (offspring of one pair) with the respective ratios in the reciprocal crosses of the two pointed mutants. There is a certain variation, with means ranging from 1 to 1.3. Table 12 shows at a glance that this variation is much smaller than in the other crosses, which, however, is not the case for the larger series in table 13 (made 3 years later). In both cases the crosses with  $svr^{poi h}$  as mother have a higher sex ratio than the reciprocal crosses, indicating at least that the two strains are not completely alike.

TABLE 13a  
SEX RATIOS IN DIFFERENT CROSSES WITH poi

Cross	2 : 1 ♂									Avg.	Total no. of flies	Avg. per bottle
	Frequency of broods											
	Below 0.8	-1 0	-1 2	-1 4	-1 6	-1 8	-2 0	Above 2				
1. (Ore × svr <sup>poi h</sup> ) × Ore....	..	6	7	7	5	3	..	2	1.21	5456	181.8	
2. (Ore × svr <sup>poi</sup> ) × Ore.....	..	4	13	7	5	..	1	..	1.18	5780	192.6	
3. (svr <sup>poi h</sup> × Ore) × Ore....	..	4	10	9	4	..	2	1	1.22	5684	189.4	
4. (svr <sup>poi</sup> × Ore) × Ore.....	..	5	6	9	7	1	1	1	1.27	6173	205.7	
5. Ore × (Ore × svr <sup>poi</sup> )....	1	9	11	6	2	1	..	..	1.11	4608	153.6	
6. Ore × (Ore × svr <sup>poi h</sup> )....	..	7	12	3	3	3	1	1	1.15	4013	133.7	
7. (Ore × svr <sup>poi h</sup> ) × svr <sup>poi h</sup> ...	1	3	18	2	3	2	1	..	1.16	5313	177.1	
8. (Ore × svr <sup>poi</sup> ) × svr <sup>poi</sup> ...	..	7	14	5	1	1	1	1	1.14	5846	194.9	
9. (svr <sup>poi h</sup> × Ore) × svr <sup>poi h</sup> ...	1	2	19	7	1	..	..	..	1.12	5954	198.4	
10. (svr <sup>poi</sup> × Ore) × svr <sup>poi</sup> ...	..	7	12	9	2	..	..	..	1.11	5255	175.1	
11. Ore × (svr <sup>poi</sup> × Ore).....	1	13	14	..	1	1	..	..	1.01	5223	174.1	
12. Ore × (svr <sup>poi h</sup> × Ore)....	2	5	17	4	1	1	..	..	1.09	5043	168.1	
13. (svr <sup>poi h</sup> × svr <sup>poi</sup> ) <sup>2</sup> F <sub>2</sub> .....	2	8	16	3	..	..	..	1	1.06	4128	137.6	
14. (svr <sup>poi</sup> × svr <sup>poi h</sup> ) <sup>2</sup> F <sub>2</sub> .....	..	6	11	7	4	..	1	1	1.19	4415	147.1	
15. svr <sup>poi h</sup> × svr <sup>poi</sup> F <sub>1</sub> .....	..	4	8	5	6	3	1	2	1.28	2926	97.6	
16. svr <sup>poi</sup> × svr <sup>poi h</sup> F <sub>1</sub> .....	1	11	9	5	2	1	..	1	1.05	3092	103.1	

TABLE 13b  
STATISTICAL TESTS OF TABLE 13a

Cross no.	Significance		Homogeneity		Broods differing significantly from 1:1	
	χ <sup>2</sup>	P	χ <sup>2</sup>	P	Number	Ratio
1	50.7	<0.01	71.7	<0.01	10	1.2-2.3
2	37.5	<0.01	48.0	<0.02	9	1.1-1.9
3	55.3	<0.01	61.5	<0.01	12	1.3-2.5
4	86.4	<0.01	71.2	<0.01	13	1.2-2.8
5	11.45	<0.01	38.99	>0.01	4	0.7-1.6
6	18.9	<0.01	44.7	<0.05	7	1.4-2.2
7	29.8	<0.01	41.8	>0.05	6	1.5-1.9
8	25.7	<0.01	44.3	<0.05	4	1.3-2.1
9	18.03	<0.01	17.43	>0.95	2	1.4
10	13.98	<0.01	27.42	>0.50	1	1.4
11	0.15	>0.50	28.71	>0.30	2	0.7-1.6
12	9.18	<0.01	38.04	>0.10	3	0.7-1.8
13	3.04	>0.05	24.56	>0.70	2	0.5-2.1
14	32.90	<0.01	43.93	<0.05	6	1.5-2.6
15	44.00	<0.01	43.86	<0.05	11	1.5-2.9
16	1.77	>0.10	33.90	>0.20	3	1.6-2.2



c) Table 12 does not give reliable information on the average number of flies in pointed broods, because the counts were made without this in mind and are therefore not comparable. But the data in table 13 were collected so that they are strictly comparable. Clearly, the average number of individuals in the outcrosses is almost twice that of the  $F_1$  between the two pointed lines.  $F_2$  of the pointed lines is, however, a little higher.

e) This is illustrated by a series of crosses, with the results shown in table 14.

f) Table 12 contains the sex ratios for a considerable number of pair crosses between females of the two pointed stocks and wild-type males, or such with different recessive markers. The variation of the sex ratios is a very considerable one. About half the broods show ratios above 1.5:1, an appreciable number even 2-3:1, and some broods contain hardly any males. The statistical analysis of the table has been made the following way. From the original data only broods with more than 50

TABLE 14  
CROSSES  $\underline{Y} \times \text{poi}$

Cross	♀	♂	Ratio
$\underline{Y} \times \text{svr}^{\text{poi}}$ .....	152	194	0.8
$\underline{Y} \times \text{svr}^{\text{poi h}}$ .....	252	311	0.8
$\underline{Y} \times (\underline{Y} \times \text{svr}^{\text{poi}})$ .....	622	617	1.0
$\underline{Y} \times (\underline{Y} \times \text{svr}^{\text{poi h}})$ .....	152	161	1.0

individuals were selected. There were 28 broods for  $\text{svr}^{\text{poi}}$  and 45 broods for  $\text{svr}^{\text{poi h}}$ . For each one  $\chi^2$  was calculated on the assumption of a 1:1 ratio. Those which differed significantly ( $P < 0.01$ ) were successively tested for the expectations 4:3, 8:5, 2:1, and 4:1, as derived from the interpretation. All those which fitted any of these expectations were subjected groupwise to a homogeneity test. Thus almost 200  $\chi^2$  were calculated, and 8 homogeneity tests. The results were:

#### $\text{svr}^{\text{poi}}$

Of 28 broods, more than 50 individuals:

- 14 fit a 1:1 ratio, and together they give a homogeneity of 9.15,  $P > 0.70$
- 14 differ significantly from a 1:1 ratio ( $P < 0.01$ ) when tested for the different expected ratios
- 11 fit a 4:3 ratio with a homogeneity 2.85 ( $P > 0.98$ )
- 13 fit an 8:5 ratio, homogeneity 7.56 ( $P > 0.80$ )
- 11 fit a 2:1 ratio, homogeneity 7.51  $P > 0.50$
- 1 fits a 4:1 ratio

#### $\text{svr}^{\text{poi h}}$

Of 45 broods, more than 50 individuals:

- 25 fit 1:1 ratio, homogeneity 17.22  $P > 0.80$
- 20 differ significantly; of these
- 17 fit 4:3 ratio, homogeneity 6.4  $P > 0.98$
- 20 fit 8:5 ratio, homogeneity 11.14  $P > 0.90$
- 17 fit 2:1 ratio, homogeneity 6.56  $P > 0.98$
- 1 fits 4:1 ratio

This indicates that the expected ratios, with considerable overlapping, are actually present, though it would be difficult to say how frequently the ratios 1.33, 1.6, and 2:1 are represented. The ratios have been derived from the assumption that two different nonreciprocal translocations from the first into the second and third chromosomes are present. The details will be discussed with the tests for individual chromosomes. In a general way we learn from this table that pointed females must frequently be heterozygous for an X chromosome that is male lethal under certain conditions which cannot be simple and which might also include balanced lethals in the extreme rates (with a little crossing over).

The backcross combinations are found in table 13, where nos. 1-6 and 11 and 12 are the combinations which do not produce pointed females, and nos. 7-10 those which segregate both pointed females and males; nos. 13-15 are controls in two generations of crosses between the pointed mutants. The series was made so as to be strictly comparable in every respect, and where the sex ratios are in favor of the females it is always the pointed male class which is deficient. Of each cross, 30 individual pair crosses were made, and the sex ratios were calculated and arranged in a frequency table with classes of 0.2. We see again a considerable variation from normal, even a slight preponderance of males, to very high ratios, though the extreme ratios recorded in table 12 for  $F_1$  are missing. Again, the frequency distribution of the different ratios indicates that the major deviations are significant. The same statistical tests were applied as before, i.e., the  $\chi^2$  for the 480 individual broods were calculated and the homogeneity test applied. Table 13a contains the results. The number of broods significantly differing from a 1:1 ratio is small when the X chromosome of the males is not derived from *poi*, also small in most of the controls and in the cases where females homozygous for *poi* could be produced and therefore lethal female classes were expected. The actual number of these significant aberrations from 1:1 is, in percentages of all broods of the respective group:

Not <i>poi</i> X chromosome in males . . . . .	13.3 per cent
Crosses with <i>poi</i> females segregating . . . . .	13.3 per cent
$F_1$ controls . . . . .	13.3 per cent
$F_2$ controls . . . . .	23.3 per cent

But in the crosses in which proper conditions for male lethal classes were present the percentage is 36.7. The homogeneity test was significantly negative for this same group but positive for all others except one (no. 5), though the  $\chi^2$  for many of these latter groups showed  $P < 0.01$ . Further analysis is hardly possible since segregation and, in many instances, crossing over must obscure the results. But in a general way the results agree with the foregoing. They also show, incidentally, the already stated nonidentity of *poi* and *poi h* (see 13-18, table 13a).

If we add all the crosses in which the *poi* X chromosome has been introduced by the maternal grandmother, paternal grandmother, etc., we get the results shown in table 15. This table is not very illuminating; nevertheless it shows clearly that the maximum of broods with male lethal classes is obtained when the mother is heterozygous (first and third group), thus giving a chance for crossing over in the X chromosome; that relatively few broods with lethal ♂ are found when the mother is not heterozygous for *poi*; that the same is true where ♀ and ♂ *poi* can be produced, i.e., eventual lethal classes in both sexes. Whereas pointed alone has a normal sex ratio, the control crosses between the two pointed are significantly different

from the other crosses, thus showing that both of them differ more or less in regard to the presence of the lethal conditions. The ratios of this table have been calculated for two limiting values of the sex ratios, 1.2 (below 1.2 : above 1.2) and 1.4 (below 1.4 : above 1.4). The latter ones show the relations more clearly, as expected. The explanation for all these ratios will be derived below and will be summarized in table 11.

TABLE 15

poi X derived from	Ratio frequencies	
	below 1.2 : above 1.2	below 1.4 : above 1.4
Maternal grandmother.....	25 : 35 = 0.7	43 : 17 = 2.5
Paternal grandmother.....	52 : 8 = 6.5	56 : 4 = 14.0
Maternal grandfather.....	30 : 30 = 1.0	44 : 16 = 2.8
Paternal grandfather.....	40 : 20 = 2.0	49 : 11 = 4.5
Both parents, i.e., ♀♂ poi.....	84 : 36 = 2.3	107 : 13 = 8.2
Control F <sub>1</sub> poi h × poi.....	12 : 17 = 0.7	17 : 12 = 1.4
Control F <sub>1</sub> poi × poi h.....	21 : 9 = 2.3	26 : 4 = 6.5
Control F <sub>2</sub> (poi h × poi) <sup>2</sup> .....	26 : 4 = 6.5	29 : 1 = 29.0
Control F <sub>2</sub> (poi × poi h) <sup>2</sup> .....	17 : 13 = 1.3	24 : 6 = 4.0

We refer again to table 5 (p. 304), which has already been discussed in relation to expressivity of the character of pointed wings. The summarized results below show a perfect regularity of the sex ratios for F<sub>2</sub> of both alleles: F<sub>2</sub> with only males pointed show the high average sex ratios 1.4 and 1.6 respectively, which illustrates again point *g* above.

*h*) The tables studied in the foregoing section indicate that the individual pointed flies are not alike in regard to their X chromosome, which might be normal or abnormal in different ways. A constitution as assumed would leave in the population different types, by segregation and crossing over, and therefore variation of type as observed is to be expected.

*i*) As not only the sex ratios but also the marker tests indicate the presence of minute translocations from the X chromosome into autosomes (no major ones are present; see salivaries, below), the standard translocation test ought to reveal them.

Actually it is completely negative. As this is an important point, some data may be given (table 16).

The table shows neither lethal nor sublethal classes. Though a Patterson stock was used which had been highly selected for an extreme and easily visible eyeless, the backcross introduced dominant modifiers which make *ey* overlap with *+*, as the addition of the complementary classes with and without eyeless shows. (See below). Moreover, controls show that the viability of the segregants decreases with

TABLE 16  
Patt  $\times$  (Patt  $\times$  poi and poi h)

Mutant	$\sigma$							
	Patt	bw e	bw ey	e ey	bw	e	ey	+
poi .....	48	57	41	43	62	59	47	63
poi h .....	14	15	12	17	24	20	14	36
	bw e $\pm$ ey		e $\pm$ ey		bw $\pm$ ey		+ $\pm$ ey	
poi .....	105		102		103		110	
poi h .....	29		37		36		50	

Mutant	$\sigma$							
	bw e ey	bw e	bw ey	e ey	bw	e	ey	+
poi .....	44	34	56	40	55	58	66	76
poi h .....	14	16	17	21	23	18	30	22
	bw e $\pm$ ey		e $\pm$ ey		bw $\pm$ ey		+ $\pm$ ey	
poi .....	78		98		111		142	
poi h .....	30		39		40		52	

$$P \sigma > 0.10 \quad P \sigma < 0.01$$

$$\text{poi h } \sigma 22.8 \quad P \sigma < 0.01 \quad P \sigma > 0.30$$

$$\chi^2 \text{ second grouping: poi } \sigma = 0.36 \quad P = 0.95 \quad \text{poi } \sigma = 20.04 \quad P < 0.01$$

$$\text{poi h } \sigma = 6.05 \quad P = 0.10 \quad \text{poi h } \sigma = 6.09 \quad P 0.10$$

the number of recessive alleles. (Controls with Oregon in table 105). Significant  $\chi^2$  values therefore may relate to such irregular features. But the numbers for poi with the correction for overlapping *ey* show a considerable difference in males and females which cannot be accounted for by these features alone (see  $\chi^2$ ) in spite of a 1:1 sex ratio. Either the viability of the classes is unusually influenced in the presence of the pointed X, or the absence of the second and third chromosomes, and, still more, second and third chromosome derived from the poi line in presence of an X from poi decreases the viability: this being the more probable explanation.

We have already mentioned the fact that lethal male classes are obtained whenever the X chromosome of sons is derived from a heterozygous mother (poi/+), and that this is the case whether this X is of grandmaternal or grandpaternal origin. This shows that lethal deficiencies in the X chromosome are compensated in the male and not (or not only) by an autosomal duplication, since  $\underline{y} \times \text{poi}$  crosses have

a normal sex ratio. But a heterozygous mother produces male lethal classes, and we must therefore assume that the compensation present in the male is absent or can be broken by crossing over. This points to a transposition of the same locus or

TABLE 17  
( $svr^{poi} \times bw; e; ey$ )  $\times$   $bw; e; ey$

♀							
bw e ey	bw e	bw ey	e ey	bw	e	ey	+
9	17	15	10	14	18	8	25
15	17	11	10	27	20	10	19
13	14	10	19	24	14	9	27
17	14	13	7	25	16	12	18
5	3	3	1	4	3	2	4
4	2	3	5	3	4	4	6
15	19	8	10	25	26	9	20
12	8	15	7	28	17	13	38
12	22	9	6	25	28	8	30
14	20	12	5	29	23	18	17
12	21	8	6	17	16	6	23
128	157	107	86	221	185	99	227

♂ + : poi each class									
bw e ey	bw e	bw ey	e ey	bw	e	ey	+	♀ : ♂	
17 3	5 5	5 10	2 1	9 2	16 3	4 3	4 5	1.38	
18 2	4 1	6 6	5 4	12 8	11 2	1 3	10 7	1.29	
9 5	5 0	10 8	4 2	5 2	4 1	5 8	16 4	1.36	
7 2	4 1	6 5	4 3	15 8	8 1	5 3	11 7	1.36	
3 2	1 ..	3 2	3 1	2 4	3 1	.. 2	6 1	0.75	
.. ..	3 2	.. 2	1 ..	.. ..	1 1	.. 1	.. 1	2.58	
14 3	3 ..	7 4	7 1	15 5	11 3	6 6	19 7	1.19	
7 2	4 ..	7 6	7 1	8 6	10 3	6 3	11 ..	1.70	
8 2	12 ..	5 8	6 2	15 3	15 ..	8 5	5 3	1.21	
10 6	5 ..	5 5	3 4	10 9	17 1	6 3	9 5	1.41	
8 2	8 ..	4 4	4 2	12 6	12 1	5 5	6 5	1.42	
101 29	54 9	58 60	46 21	103 53	108 17	46 42	97 45	1.36	
Σ.....	130	63	118	67	156	125	88	142	
Ratio + : poi.	3.5	6	0.9	2.1	2	6.3	1.1	2.1	

$$\chi^2 \text{ } \varnothing = 142.53 \quad P < 0.01$$

$$\chi^2 \text{ } \sigma = 75.28 \quad P < 0.01$$

$$\chi^2 \text{ sex ratio for } 4 : 3 = 0.34; P > 0.50; \text{Homogen. } P > 0.70$$

loci within the poi X to a considerable distance away from the deficiency, in addition to translocation into an autosome. First information on this point ought to be derived from similar crosses involving marked autosomes but introducing the poi X via a female. This leads to point *k* of our enumeration.

*k*) Tables 17 and 18 contain the results of the backcrosses ( $svr^{poi} \times bw, e, ey$ )

× bw, e, ey. (The controls with Oregon are found in table 105.) Table 17 shows first a considerable deviation from a 1:1, etc., expectation (see  $\chi^2$ ) in both sexes. Looking over the data, one realizes at once that different features are involved: first, the already encountered suppressor action for eyeless more extreme here than before; secondly, the abnormal sex ratio; thirdly, definite features with respect to the ratios of plus:poi males which deviate from the expected ratios (poi does not mean the phenotype pointed wings which is dependent upon enhancers, but actually all poi pale =  $svr^{po1}$  males).

TABLE 18  
(Like table 17, but ey and not ey classes combined)

♀				♂ + : poi each class			
bw e ± ey	bw ± ey	e ± ey	+ ± ey	bw e ± ey	bw ± ey	e ± ey	+ ± ey
				22 8	14 12	18 4	8 8
				22 3	18 14	16 6	11 10
				14 5	15 10	8 3	21 12
				11 3	21 13	12 4	16 10
				4 2	5 6	6 2	6 3
				3 2	.. 2	2 1	.. 2
				17 3	22 9	18 4	25 13
				11 2	15 12	17 4	17 3
				20 2	20 11	21 2	13 8
				15 6	15 14	20 5	15 8
				16 2	16 10	16 3	11 10
285	328	271	326	155 38	161 113	154 38	143 87
Σ.....				193	274	192	230
Ratio + : poi.....				4 05	1.42	4.05	1.63

$$\chi^2 \text{ ♀} = 8.29 \quad P = 0.05$$

$$\chi^2 \text{ ♂} = 20.32 \quad P = 0.01$$

$$\chi^2 \text{ not e classes for exp. } 4 : 3 = \text{♀ } 0.34 \text{ } P > 0.50, \text{ ♂ } 2.38 \text{ } P > 0.10$$

The situation for eyeless suppression becomes clear when we compare the reciprocal classes with and without ey; e.g., bw, e, ey : bw, e, etc., and when we compare the added reciprocal classes with each other, which is done in table 18. The females show a simple result. The classes plus: ey, bw : bw, ey and e : e, ey all show a ratio of approximately 2:1, and the classes bw, e : bw, e, ey, a ratio of 1:1. This shows clearly that the second and third chromosomes of poi contain a suppressor action for eyeless with such an effect upon about 1/3 of the eyeless males. Correspondingly, when the not eyeless and eyeless groups are added (see table 18) almost normal ratios of the groups appear. Special tests were made to prove this interpretation. They will be reported later when a corresponding but more extreme action in a different allele will be reported.

But still the groups containing e/e are smaller than those with e/+ with perfectly regular numbers, in both groups, and a ratio of 1.17. This requires that a third chromosome from poi enhance the viability of the females whether a second one is present or not. We do not know what this action is, but as small translocations into the second and third chromosomes of poi will be demonstrated, these are suggested

as being responsible for the suppressor effect as well as the viability effect. In all classes the first chromosomes are either *poi/+* or *+/+*, which might mean that the actions are confined to the unbalanced combinations with normal first chromosomes. We may add that recently Pontecorvo (1943) came to the conclusion that lowered viability in partial hybrids of two *Drosophila* species is based upon very small translocations.

In the males (tables 17, 18) the situation is complicated by the presence of male lethal classes in the pointed groups. If we look at only the *ey* suppression we can distinguish here between males with and without the *poi X*. In those without *poi X* no lethal classes are involved and actually the added *ey* and not *ey* groups contain equal numbers of individuals of about  $\frac{1}{2}$  of the female classes (see table 18, summation). The *ey* suppression in not *poi* ♂ is the same in the *plus*, *bw*, *e* classes as is found in females (ratio 2 not *ey* : 1 *ey*), pointing to the explanation given above. But there is a strange ratio observed in the *bw*, *e*, *ey* : *bw*, *e* classes, the latter being half the size of the former. As both added have the expected number, we see in the males without a *poi X* now the opposite of *ey* suppression, namely, a dominance effect of *ey* in about  $\frac{1}{3}$  of *bw*, *e*, *ey/+* ♂. As this result was not obtained in females (with actually more *bw*, *e* ♀ than *bw*, *e*, *ey* ♀), the *X* chromosome must be involved via crossing over, i.e., in the presence of an *X* without the *poi* end, but in the presence of other parts from *poi* to the right of this locus the dominance effect upon *ey* occurs even with second and third chromosomes not derived from *poi* stock. If this is the case, the responsible locus in the *poi X* would be located 30 units to the right of *poi*. One would like to think of a Dubinin effect involving a translocation  $4 \rightarrow 1$ . But as this does not tally with the *ey* suppression effect which is present in both females and males only in the presence of a second or third chromosome from *poi*, it seems more probable that no translocation  $4 \rightarrow 1$  is involved, but a specific dominance-enhancing effect of a point in the *X* chromosome. If our assumption is correct that the suppressor effect is due to the duplications by translocation  $1 \rightarrow 2$  and  $1 \rightarrow 3$ , this might mean that the same duplication in the form of a transposition acts as a dominance enhancer. This might be checked by a comparison with the Patterson crosses (table 16) in which the females have always non-*poi X* chromosomes but the same autosomes as in the present case, whereas all the males have a *poi X* chromosome without possibility of crossing over. Actually, the eyeless suppression is here rather small in both sexes though larger in the females, and the female classes added from eyeless and not eyeless show a perfect 1 : 1 : 1 : 1 ratio, whereas the males have very poor ratios. These data thus agree only in part with the conclusions just derived. Again, we shall return later to the same problem in connection with another *poi* allele which shows the dominance effect in extreme form.

We turn now to the more important problem, the distribution of male defective classes, which, as we saw, are limited to the group with the *X* chromosome containing *poi*, though such classes are always absent in the  $\underline{y} \times \textit{poi}$  crosses with all males possessing a *poi X*. We see from table 17 that the entire sex ratio is near 4 : 3 (1.36), and as we know that the not *poi* males are all present,  $\frac{1}{2}$  of the *poi* males are missing. But these are not equally distributed among all the autosomal classes, as the lower end of both tables shows. Actually the ratios are, if the correction for *ey* suppression is made (table 18), near 4 : 1 in both classes containing *e/e*, and near 1.5 : 1 in both classes with *e/+*, whether either is *bw/bw* or *bw/+*. When the correction for *ey* sup-

pression is not made (table 17), the ratios for  $e/e$  and  $bw/bw$ ,  $e/e$  are near 6:1, those for  $e/e$ ,  $ey/ey$  with  $bw/bw$  or  $bw/+$ , near 3:1; those for  $e/+$   $ey/+$  with  $bw/bw$  or  $bw/-$ , 2:1; and those for  $ey$  with  $bw/bw$  or  $bw/+$ , 1:1.

The individual broods recorded in table 18 show that the high ratio of poi lethality is consistently present in all individual broods and therefore requires a single causation. As the majority ( $\frac{3}{4}$ ) of poi males in these groups are lethal, it is obvious that the poi X in the absence of proper third chromosomes ( $e/e$  class) is lethal except in  $\frac{1}{4}$  of the individuals, a fact which must be due to crossing over, as the other autosomes do not influence the result (taking into account the  $ey$  suppression). As the numbers of the not poi classes are exactly those expected from the female numbers (i.e.,  $\frac{1}{2}$ ), the crossing over cannot have occurred in the X chromosome, since otherwise the same number of lethals would occur in the not poi class as there are survivors in the poi class. Therefore crossing over in the third chromosome between the locus of  $e$  and the locus of a nonreciprocal translocation  $1 \rightarrow 3$  would account for the facts. If this is the case, equal numbers of crossover gametes containing  $e$  and the T and  $+$  without T are formed. The latter are found in the backcross classes  $e/+$ , where, therefore, the same percentage of poi males must be missing as survive in the  $e/e$  classes. Actually, 25.4 per cent survive in the  $e$  classes (which would be the crossover value  $e-T$ ) and 34.2 per cent are missing in the  $e/+$  classes. This is roughly according to expectation,  $\chi^2$  of the not  $e$  classes for an expectation of 4+:3 poi is not aberrant, and the homogeneity test for all classes is positive. In spite of this, a comparison of the individual broods for the  $e/e$  classes shows very little variation, whereas the  $e/+$  classes seem to contain two major types, one nearer 1:1, the other nearer 2:1. Thus probably there is involved in the ratios something else, which is not clearly visible here but seems to result from the fact that the individual broods are not alike. This means that different  $F_1$  combinations exist, which again requires that the original poi female be heterozygous for something in one of the chromosomes. The details of an analysis of individual chromosomes now to be presented will point out such possibilities.

*Tests for individual chromosomes: the second chromosome.*—The following crosses are made with second chromosomes marked with  $ll$  sp. In the presence of homozygous or hemizygous pointed, speck is suppressed (completely in males, sometimes incompletely in females.) The  $ll$  (sp) class therefore is  $ll$  sp + poi (crossing over being only a fraction of 1 per cent. In  $F_2$  both ♀♀ and ♂♂ of this combination are expected, and in the backcross only males. Table 19 shows a rather normal segregation for +, poi, and  $ll$  sp (7-8 not  $ll$  sp:1  $ll$  sp in  $F_2$ , 2 not  $ll$  sp:1  $ll$  sp in  $R(F_2)$ ), but only a small number of  $ll=ll$  sp+poi. Either this class is semilethal, or it is completely lethal except for the crossovers between pointed and a lethal deficiency by translocation in the X chromosome (translocated into the second chromosome) and the crossovers between  $ll$  and the duplication in the second. If we call  $q_2$  the crossover value between  $ll$  and the duplication in the second chromosome, and  $q_1$  the crossover value between  $svr^{po}$  and the deficiency in the first, the expected  $F_2$  ratios for the males are:

$$300 + : 300 - \frac{q_2}{2} \text{ poi} : 100 - \frac{q_1}{2} \text{ ll sp} : \frac{q_1 + q_2}{2} \text{ ll (sp) poi}$$

This assumes that everything is normal except the translocation in question. Among the crosses reported in table 19 the second one clearly represents this situation,



since we have a normal sex ratio in the three large classes. The numbers of + and poi classes are not equal; the reason is that the classification had been made for the wing character alone, which is modifiable as reported above. In the last two broods the classification is a perfect one, in no. 4 by marking the tip of the poi chromosome with w, in no. 5 by checking for the pale color; and the two classes are equal. The ratio of + (and poi) : ll sp not poi ought to be near 6 : 1. It is actually much higher in most of the crosses; this indicates a lower viability of the segregating ll sp class or a further complication. The class of ll (sp) poi contains only a few males but more

females. An exact calculation of  $\frac{q_1 + q_2}{2}$  is prevented by the imperfect ratios for ll sp, but an estimate based upon the formula given above and the numbers for cross 2 would give a value for  $q_1 + q_2$  of about 8 per cent for the males, i.e., both points would be rather near the markers. For the first chromosome we have the additional

TABLE 19

Cross	Broods	+		poi		ll sp		ll poi		Sex ratio
		♀	♂	♀	♂	♀	♂	♀	♂	
1. $F_2$ (svr <sup>poi</sup> × ll sp) <sup>2</sup> .....	8	586	586	216	51	103	44	26	2	1.36
2. $F_2$ (svr <sup>poi h</sup> × ll sp) <sup>2</sup> .....	9	678	675	201	191	117	101	21	6	1.04
3. $RF_2$ (svr <sup>poi</sup> × ll sp) × ll sp...	2	179		187		133	81	...	2	1.15
4. $F_2$ (svr <sup>poi h</sup> w × ll sp) <sup>2</sup> .....	1	26	30	26	26	3	3	3	5	0.9
5. $F_2$ (svr <sup>poi</sup> × ll sp) <sup>2</sup> .....	1	17	17	17	18	9	2	4	..	1.28

information furnished by cross no. 4. Here only the tip of the X chromosome from poi containing svr<sup>poi</sup> is present, the rest being replaced to a point between w and svr. The two ll sp classes are equal, showing the deficiency to be located beyond the break between svr and w. A surprising fact is that a much larger number of ll poi females are recorded than males. It turned out (testing with ll sp) that they were actually mostly ll sp/+; poi/poi, ♀ with an unusually high grade of pointed which cannot be distinguished from a lower-grade ll; thus only the male numbers are reliable.

Backcross no. 3 is in agreement (expectation ♀♀, + : ll sp = 1 : 1, ♂♂, + and poi : ll sp : ll sp poi = 2 : 1 : c.o.). In the first  $F_2$  some additional feature is present. One-fourth of the males are missing. If we assume that the plus class contains also a part of the pointed class with inhibition of the pointed character (see above), we expect 401 females 401 males +, 401 females poi; thus we actually have 236 males, poi, i.e., 41 per cent of the males are missing. In ll sp without poi more than 1/2 of the males are missing. If we assume that the translocation from X to the third chromosome found before was present in these crosses (together with that for the second chromosome), and if the locus of the deficiency in the first chromosome is near svr, we expect 1/4 of the poi males to be missing. But in ll sp without poi only the crossovers in the first chromosome between svr and the deficiency would be missing among 1/4 of the class. Thus something else affecting the males must be present. If there were a transposition in the X in addition to the two deficiencies, and the deficient point were far away from svr but the transposed section nearer it, a small

percentage of lethal male combinations would be added to the poi class (double crossover with poi and the far-away deficiency), and a larger number of not poi males would be lethal by crossing over between insertion and deficiency. The data hardly permit a decision except that the number of 11 sp males is still too low for such an assumption.<sup>4</sup>

TABLE 20  
(svr<sup>poi</sup> × a) × a

No.	♀		♂			a poi
	+	a <sup>a</sup>	+	poi	a	
7979 B .....	99	72	45	27	54	1
7982 B .....	62	45	11	4	22	1
7983 B .....	80	82	36	14	34 <sup>b</sup>	..
7985 B .....	88	95 <sup>c</sup>	36	33 <sup>d</sup>	63	1
7986 B .....	88	65	43	8	27	3
7988 B .....	81	67	43	26	37	..
Sa.....	498	426	214	112	237	6(?)
	♀ +	♀ a	♂ +	♂ poi	♂ a	♂ a poi
Expected.....	462	462	231	231	231	231
Found.....	498	426	214	112	237	6

<sup>a</sup> a = arc.

<sup>b</sup> 5 ♂ spoon-like blist wings.

<sup>c</sup> 1 ♀ blist.

<sup>d</sup> 1 ♂ blist.

TABLE 21

Cross	+	♂		a poi
		poi	a	
(svr <sup>poi h</sup> × a) × a.....	176	81	177	9 (?)
(svr <sup>poi</sup> × a) × a.....	186	82	182	15 (?)

We continue with the second chromosome. Table 20 contains a set of crosses (svr<sup>poi</sup> × a) × a which were made in 1935 soon after svr<sup>poi</sup> had appeared. Table 21 contains a repetition, made six years later for both pointed alleles, giving only the sum total of the males. It is obvious that the ratio of 8 ♀♀ : 5 ♂♂ = 1.6 found in both cases is based upon the absence of 1/2 of the pointed males and of almost all of the arc pointed males. (The females are as expected.) The arc locus (or region) of the poi stock is therefore needed if the poi X is present in males, which again points to the nonreciprocal translocation from the poi chromosome to the right end of the second. In addition, another condition makes 1/2 of the poi males disappear, which is clearly the already studied translocation 1→3. (Actually a, poi [a pale or a px (sp) pale] can be isolated and bred if a pointed stock without the translocation is used.) Again, a small number of a, poi males survive marked with (?). The reason is the already

<sup>4</sup> It was found later that the 11 sp stock contained a homozygous lethal inversion in the third chromosome. But this cannot be involved in the aberrant results, since it affects all segregating classes.

known weak dominance effect of arc in crosses with pointed. Therefore the number of arc poi males might also contain some of these heterozygotes. This makes an exact calculation of the crossover value impossible. At best it must be very low (see the formula given above). Probably the locus of the translocation is very near arc, and the few survivors are the crossovers in the first chromosome.

TABLE 22  
(svr<sup>poi</sup> w × a px sp) × a px sp  
(Only ♂; px sp neglected, but a poi checked by sp suppression)

Sa.	a	+	svr <sup>poi</sup> w	a svr <sup>poi</sup> w
1.....	13	14	17	11
2.....	11	14	9	12
3.....	18	10	10	9
4.....	15	23	19	17
Sa. 1-4.....	57	61	55	49
5.....	24	19	9	6
6.....	22	19	15	8
7.....	17	27	10	9
Sa. 5-7.....	63	65	34	23

A first check upon this result is, as in the case of ll sp, the use of a pointed in which the part to the right of the X chromosome including white has been replaced by crossing over, i.e., the combination svr<sup>poi</sup> w. If the deficiency by translocation is located to the right of white the lethality with a/a ought to be absent. Table 22 (only males registered) contains the answer. It is obvious that the almost complete lethality of a/a, poi males is here absent. Two groups of ratios are visible. The first (1-4) is practically normal. In the second (5-7), 1/2 of the pointed males are miss-

TABLE 23  
(a px sp × svr<sup>poi</sup>) × a px sp

+	♀					♂			C.o. and poi sp	Sex ratio
	a px sp	a	a px	sp	+	a px sp	poi	a px		
248	207	1	6	18	108	92	61	22	1	1.7

ing in both classes, a result which we saw before as being independent of the second-chromosome translocation, actually based upon the translocation 1→3 reported above. It turned out in this and many other crosses that pointed individuals from stock might contain one or the other, or both, or none, of these translocations.

Other checks were made, involving markers in the right end of the second chromosome, which permit a check on crossing over. In one of these the pointed X was introduced via a pointed male; the result is summarized in table 23, which again shows the sex ratio 1.7 based upon both translocations 1→2 and 1→3. The female crossover classes are about the expected ones (distance a-px 1.3, px-sp 6.5). In the

males crossovers a px with or without poi and a px sp, poi with sp suppression were not clearly distinguished. But if we estimate the crossovers from the normal expectation (not forgetting the lethal classes), only a small proportion of apx (sp) poi males remain, as in the other examples. In view of this similarity the translocation ought to be to the left of arc. Two other sets using the markers a sp and px bw sp gave essentially the same results, though a higher crossover value, i.e., survival of more pointed males with foreign second chromosomes was found. This point will be discussed below (see table 28). Also, reciprocal  $F_2$  were used, in one of which lethal female classes are expected. The segregation again showed the same phenomenon and also a higher crossover value for the X chromosome, namely, about 10-12 per cent. But admittedly the calculations are not very reliable.

Only one more test, with quintuple = b pr vg a sp, will be mentioned, because it was made immediately after the appearance of pointed (table 24). It shows that at that time the translocation 1→3 was not present in the tested individuals, but the translocation 1→2 was there (sex ratio 1.36). It must be kept in mind that poi suppresses sp and that the flies containing vg which do not show the pointed wing character had not been checked for pale color. Flies with arc may be arc or a (sp) with poi. According to expectation, plus males are  $\frac{1}{2}$  poi, and among the quintuple flies nearly  $\frac{1}{2}$  with poi are missing. (A part of the 5 ple not poi males will be crossovers poi→Df.) The crossover classes with b and pr contain  $\frac{1}{2}$  pointed flies. The classes containing arc have only  $\frac{1}{2}$  of the males, the pointed males being lethal. The class arc alone gives information upon the surviving a poi males. Crossover males ought to equal the females in number; the rest are survivors a sp and poi. As there are 123 a sp females, about 60 males a sp poi are expected. Twenty survivors, i.e., about 33 per cent, is an unusually high percentage. The class b pr vg (a), in which arc cannot be distinguished, contains 140 females, 107 males, the missing males being those containing arc and poi. The number agrees with the expectation if the translocation is between vg and a near the latter (vg-a = 22.2).

One of the broods in table 24 is very strange and aberrant, namely, no. 6474. Here all plus males are poi, which requires a condition in an autosome, lethal if poi is not present (in addition to the known translocation). In b pr vg (a) most males are missing, and all males, not only those with poi, in the a sp and sp classes. The latter would be the combination of the plus lethal with the poi lethal condition. The result is a very high sex ratio, 146 : 62 = 2.3. The numbers of the a sp class seem to put the disturbance in the second chromosome between vg and a. The situation never recurred so as to permit of an analysis.

We reported that in later tests  $svr^{poi}$  and  $svr^{poi^h}$  gave the same results for the second chromosome and frequently also those expected for the 1→3 translocation, or both. Soon after the appearance of poi (1935),  $svr^{poi^h}$  was tested in the same way (nos. 7737-43, 7746-51 B). Among 10 backcrosses with the markers a sp and px bw sp, 8 contained the translocation from the first to the second chromosome (a px region) with the consecutive sex ratios, e.g., classes a sp and px bw sp.: 283 ♀ 125 ♂. In addition, there were 46 ♂ px bw (sp) and a (sp) and 11 such females, i.e., in 35 males with pointed in two broods the translocation was absent as far as these data go, or at least not present with certainty. There was no clear indication of an otherwise lethal poi class (though it was found in other similar crosses), which then, it seems, was only selected out later in this stock (see below: the third chro-



TABLE 25  
(poi × triple) × triple

Class	svrpoi			svrpoi h			Sex ratio		σ <sup>a</sup> + : 1 σ <sup>a</sup> poi	
	♀	σ <sup>a</sup>		♀	σ <sup>a</sup>		svrpoi	svrpoi h	svrpoi	svrpoi h
		+	poi		+	poi				
+	62	27	20	82	33	17	1.32	1.64	1.35	1.94
ru h st p <sup>a</sup> ss e <sup>a</sup>	34	22	6	48	35	1	1.21	1.33	3.67	35.0
ru h st p ss e	18	8	7	24	13	14	1.20	0.9	1.14	0.93
ru h st p ss e	29	7	2	21	9	..	3.22	2.33	3.5	∞
ru h st p ss e	17	9	3	23	19	6	1.42	0.9	3.0	3.16
ru h st p ss e	17	6	3	27	11	1	1.89	2.25	2.0	11.0
ru h st p ss e	2	..	..	..	3	1				
ru h st p ss e	3	..	..	2	..	..				
ru h st p ss e	3	4	..	7	1	1				
ru h st p ss e	6	2	..	5	5	..				
ru h st p ss e	11	5	2	8	9	1	1.43	0.8	2.5	9.0
ru h st p ss e	13	8	4	17	10	..	1.08	1.7	2.0	∞
ru h st p ss e	5	..	..	1	..	..				
ru h st p ss e	5	..	..	3	4	1				
ru h st p ss e	1	..	..	..	..	..				
ru h st p ss e	1	1	..	1	..	1				
ru h st p ss e	1	..	..	..	..	..				
ru h st p ss e	1	..	..	..	..	..				
ru h st p ss e	2	3	..	..	2	..				
ru h st p ss e	2	2	1	2	..	..				
ru h st p ss e	..	..	..	1	1	..				
ru h st p ss e	..	..	..	1	1	..				
ru h st p ss e	2	..	..	3	..	..				
ru h st p ss e	2	4	2	3	1	..				
ru h st p ss e	2	1	2	2	1	2				
ru h st p ss e	3	2	1	3	..	..				
ru h st p ss e	6	3	2	4	3	1				
ru h st p ss e	2	4	..	5	..	..				
ru h st p ss e	1	2	..	7	5	..				
ru h st p ss e	..	1	..	..	..	..				
ru h st p ss e	..	2	..	1	..	..				
ru h st p ss e	..	..	1	3	1	..				
ru h st p ss e	1	..	..	..	..	..				
ru h st p ss e	..	1	..	..	..	..				
ru h st p ss e	..	1	..	..	..	..				
ru h st p ss e	..	..	..	1	..	..				

mosome). Other localization tests made with other pointed alleles all agreed with a localization of the translocation near and to the left of arc.

We remember that F<sub>1</sub> poi × arc always showed a slight dominance effect of arc not observed otherwise. If the translocation into the second chromosome is actually located next to arc, as the salivaries indicate (see below), the arc segment might be involved and the compound effect a/T might actually be a position effect in this segment. We further reported above the presence of a dominant enhancer for pointed wings in this stock, and that we found a better expression of pointed wings

in males from the cross  $\text{poi} \times N$  when  $N$  contained arc. There is a possibility that this enhancer is identical with the translocation, but an exact test would be very difficult to perform and was not tried.

*Tests for individual chromosomes: the third chromosome.*—We found in the analysis of the second chromosome that in addition to the translocation  $X \rightarrow 2$  near arc, which produced deficient male lethal classes, another condition was present in

TABLE 26  
CROSSING OVER TABLE 25

C.o.	♀	♂	Standard
ru-h.....	24.9	24.0	26.5
h-st.....	22.0	22.2	17.5
st-p <sup>h</sup> .....	3.2	2.6	3.5
p <sup>h</sup> -ss.....	6.8	9.0	10.5
ss-e <sup>a</sup> .....	16.4	16.2	12.2

some of the crosses which eliminated also  $\frac{1}{2}$  of the normal males. A similar translocation into the third chromosome from the first was assumed on the basis of former data (the bw, e, ey crosses) which had demonstrated such a nonreciprocal translocation. Those earlier data permitted a rough estimate of the localization which will now be tested with marked third chromosomes. Table 25 contains the summarized results for three backcrosses of  $\text{svr}^{\text{poi}}$  and  $\text{svr}^{\text{poi}^h} \times \text{triple (ru h st p}^h \text{ ss e}^a)$ , all made soon after the appearance of  $\text{svr}^{\text{poi}}$ . Crossing-over values are rather high in some

TABLE 27  
RATIOS FOUND IN THE TRIPLE BACKCROSS, TABLE 25

	$\text{svr}^{\text{poi}}$			$\text{svr}^{\text{poi}^h}$			Sex ratio		$\sigma^+ : 1 \sigma^h \text{ poi}$	
	♀	$\sigma^+$	$\sigma^h \text{ poi}$	♀	$\sigma^+$	$\sigma^h \text{ poi}$	poi	poi h	poi	poi h
All small classes with e...	25	17	4	24	10	..	1.24	2.4	4.25	$\infty$
All small classes without e	26	16	5	31	18	7	1.24	1.24	3.20	2.57
All classes with e.....	118	60	19	137	75	2	1.49	1.78	3.16	37.5
All classes without e.....	134	65	37	168	92	55	1.31	1.15	1.76	1.67
All classes ru, not h, not e.	18	11	7	24	13	14	1.0	0.9	1.57	0.93
All classes h, not ru, not e.	8	3	2	13	11	3	1.6	0.9	1.50	3.66
All classes ru h, not e.....	34	18	5	38	32	9	1.48	0.97	3.6	3.56

classes (see table 26), though not much significance is attached to this (aside from corrections for lethal classes).

There is a considerable difference between the two groups of crosses. In all crosses with  $\text{svr}^{\text{poi}^h}$  there is a clear difference between reciprocal crossover classes: all not containing ebony have an approximately normal sex ratio, those with ebony have a high sex ratio (for this and the following see the ratios at the right side of table 25 and the summarized ratios in table 27). The ratios of plus : poi h males are extremely high in the presence of ebony; actually there is practical lethality of e, poi h males. As nearly all classes with e from triple to e alone are almost devoid of poi h males, whereas the proper number of plus males are present, the insertion is probably

located to the right of *e* (70.7). A little information is given by the double (and more) crossover classes without *e*, which will often contain the insertion except when there is another crossover break between *e* and the insertion. Actually these classes (table 27) contain *poi h* males, but less than half the expected number, which roughly agrees with a localization far to the right of *e*. The few *e, poi h* males indicate that the deficiency in the first chromosome is located very near the pointed locus, the few *poi* males being the crossovers between *poi*→*Df*. In the not ebony classes the  $+$ :*poi h* male ratio is normal in some crossover classes, high in others. This latter fact, however, has a different significance. We remember (see p. 304, above) that a dominant modifier for the expression of the pointed character (wing) is located in the third chromosome. Thus, whenever there is a normal sex ratio but too few pointed phenotypes it has to be assumed that this phenotypical modification is involved wherever the classification was made for the wing character, which was the case in the earlier work. In all these classes with normal sex ratio (not *e*) about  $\frac{1}{2}$  of the males are visibly pointed when *ru* is present but not *h*, whereas in the classes *h, not e, not ru* a normal sex ratio combines with almost  $\frac{4}{5}$  of the males not *poi*. The same is true for classes *ru h not e*. The enhancer is thus localized between *ru* and *h* at the left end of the third chromosome (see table 27).

In the noncrossover classes, especially the triple class, the numbers are less good. A comparison with the plus class shows that the triple females are less viable, which makes for a too low sex ratio. If this were corrected, the class would agree with the other results. In the plus class as well as in the crossover classes the ratio  $+$ :*poi* ♂♂ is nearer 2:1 with a sex ratio near 4:3, which is expected if the second-chromosome translocation is simultaneously present.

In this set, made shortly after the appearance of *svr<sup>poi</sup>*, this allele gave different results from those obtained for *svr<sup>poi h</sup>*. The summarized data are rather irregular. The individual data seem to indicate that one backcross behaved like *svr<sup>poi h</sup>* and that the others lacked the third chromosome translocation, which together makes for a result between the one discussed and normalcy. But the individual numbers are too small for a reliable statement. Tests made later with the *svr<sup>poi</sup>* stock revealed that the third-chromosome translocation was frequently absent (see below for other alleles), but that the intensifier was present, which latter again turned out to be located between *ru* and *h*. All crossover classes with the right end of the third chromosome derived from the marker stock contain 99+:33 *poi*; the reciprocal classes with the right end from *poi* third chromosome 49+:50 *poi*. As the classes with fewer *poi* males are even larger than the equal ones no lethal class is involved. Altogether the results reported for the second and third chromosome indicate that the 1→3 translocation is sometimes present and sometimes not. It seems that originally it was more frequent in *svr<sup>poi h</sup>*, but this might have been purely accidental, depending upon the individuals selected for breeding from a mixed stock.

Years later, the end of the third chromosome beyond ebony was tested again for the translocation with the markers *st sr e<sup>+</sup> ro ca* (*e<sup>+</sup>* ebony 70.7 *ro* rough 91.1 *ca* claret 100.7). In all crossover classes there were as many pointed as not pointed males, with the exception of the class *st sr e<sup>+</sup> ro* (the reciprocal class *ca* being normal), in which there were almost no *poi* males, the number being statistically significant. This puts the translocation between rough and claret in agreement with former data.



*Some additional data for the second and third chromosomes.*—The data on the second chromosome showed that in different tests made at different times two types of result appeared (see p. 323). In one group the combination of homozygous foreign second chromosomes with the pointed X was practically lethal except for a few survivors by crossing over. But in the original records these crossover individuals showing arc had received an interrogation mark, and, as the  $F_2$  tests showed, certainly some of them were not reliably classified (in this case because of the heterozygous arc effect). Thus the insertion is supposed to be practically at the arc locus and the deficiency rather near the svr locus. In another set of experiments a much higher rate of survival was found, with rather consistent values. Table 28 summarizes these values (part of them taken from preceding tables).

TABLE 28  
FURTHER CROSSES *poi* × SECOND-CHROMOSOME MARKERS

Cross	$\sigma^7$ marker not <i>poi</i>	$\sigma^7$ marker and <i>poi</i>		Percentage of survivors
		absolute	corrected <sup>a</sup>	
(a px sp × svr <sup>poi</sup> ) × a px sp . . . . .	92	22	16	17.4
(5 ple × svr <sup>poi</sup> ) × 5 ple . . . . .	52	26	20	38.4
(a sp and px bw sp × svr <sup>poi</sup> ) × a sp . . . . .	78	30	22	28.2
(px bw sp × svr <sup>poi</sup> ) × px bw sp . . . . .	115	49	45	39.1
(a px sp × svr <sup>poi</sup> ) <sup>2</sup> . . . . .	24	7	7	29.2
(svr <sup>poi</sup> × a px sp) <sup>2</sup> . . . . .	59	21	12	20.3
Sa . . . . .	420	122		29

<sup>a</sup> The correction consists in subtracting second-chromosome crossovers of the same phenotype found also in the ♀ and thus expected to be contained also among the *poi* ♂.

These facts (those reported above and those considered here) suggest the hypothesis that in some pointed individuals only the translocation 1→2 is present, with the deficiency in the first chromosome near the svr locus; and that in other individuals a duplication for the same region is present somewhere else, which might be a second translocation of the same section near *poi* into the third or a transposition within the X chromosome. We shall see when studying the first chromosome that the facts agree with the latter assumption. In the presence of the additional 1→3 translocation one-half of the combinations with pointed is expected to be missing even if the deficiency by translocation 1→2 is compensated by a duplication. If a transposition within the X chromosome is responsible for this duplication, the noncrossovers between the deficiency and the transposition will be the survivors, but again half will be lethal because of the 1→3 translocation. Thus a crossover value within the transposition of about 20 per cent would be derived from table 28. We shall find the possible locus somewhere near 33 in the first chromosome.

But there are some difficulties in this explanation. It is true that pointed males with the foreign second chromosome were almost completely absent in a series of crosses in which a female had introduced the *poi* X. Such a female may be heterozygous for two types of X. In the crosses tabulated in table 28, however, the *poi* X was introduced in all but one via a male, which must have had compensation for the

deficiencies, as the translocation tests and the crosses with  $\underline{y}$  had shown. This is in agreement with the transposition hypothesis. But here is a difficulty: the first set showed clearly another lethal condition ( $\frac{1}{2}$  males poi in the plus class missing), which we assumed to be the translocation 1→3. This again requires for the normal males used in the translocation and the  $\underline{y}$  tests a duplication for this translocation, as no males were missing and no compensating female lethal class could be expected. The present data (not contained in table 28) show that no pointed plus males are missing. This must mean that in this set the third-chromosome translocation was absent, and therefore no conclusion upon an eventual transposition for this translo-

TABLE 29  
EXPECTED RATIOS

Autosomes of F <sub>1</sub> mother		X chromosome of RF <sub>1</sub> sons	Sex ratio, c.o. class with DF	Sex ratio, reciprocal c.o. classes, one with DF
2d	3d			
Normal	Normal	DF tr.→2	1 : 0	2 : 1 = 2
Normal	Normal	DF tr.→3	1 : 0	2 : 1 = 2
Normal	Normal	Both DF	1 : 0	2 : 1 = 2
Heterozygous insertion	Normal	DF tr.→2	2 : 1	4 : 3 = 1.33
Heterozygous insertion	Normal	DF tr.→3	1 : 0	2 : 1 = 2
Heterozygous insertion	Normal	Both DF	4 : 1	8 : 5 = 1.6
Normal	Heterozygous insertion	DF tr.→2	1 : 0	2 : 1 = 2
Normal	Heterozygous insertion	DF tr.→3	2 : 1	4 : 3 = 1.33
Normal	Heterozygous insertion	Both DF	4 : 1	8 : 5 = 1.6
Heterozygous insertion	Heterozygous insertion	DF tr.→2	2 : 1	4 : 3 = 1.33
Heterozygous insertion	Heterozygous insertion	DF tr.→3	2 : 1	4 : 3 = 1.33
Heterozygous insertion	Heterozygous insertion	Both DF	4 : 1	8 : 5 = 1.6

DF = deficiency; tr.→ = translocated into.

cation could be drawn in these experiments. We shall return to this problem when discussing the first chromosome.

*Tests for individual chromosomes: the first chromosome.*—The complicated expectations for the first chromosome are contained in the foregoing discussion. The decisive points of that analysis are: (1) A very small section of the X chromosome has been translocated into the right end of the third chromosome. The resulting deficiency is expected to be rather closely linked with  $\text{svr}^{\text{poi}}$ . (2) Another small section of the X chromosome has been translocated into the second chromosome in the arc region. The crossover values indicated again a position in the X chromosome near  $\text{svr}^{\text{poi}}$ . (3) The fact that translocation tests were negative when unbroken X chromosomes (no crossing over) were involved requires not only that the deficient portions of the X be translocated into the second and third chromosomes, but also that they be transposed into the right part of the X itself. No other interpretation of all the facts seems possible, as the participation of the Y chromosome can be ruled out (normal sex ratios in F<sub>1</sub> and F<sub>2</sub> of  $\text{XXY} \times \text{poi}$ ). The probable locus of the transposition of the piece otherwise translocated into the second chromosome was about 20 units from  $\text{svr}$ . (4) The individuals of the pointed stocks did not all appear alike. It seems that the deficiencies in the first chromosome were not always present; actually that either, both, or none of the translocations might be found in

TABLE  
 $X^4 \times SVT^{pool} \quad X^1$

Class	Number/broods																Total		Total						
	102/3		131/2		138/2		T 7/2		120/1		125/1		T 1/1		T 2/1		T 3/1			T 8/1		T 9/1		♀	♂
	77	81	83	53	101	70	62	39	31	26	32	26	41	21	17	6	33	23		21	18	0	2	501	365
ct <sup>6</sup> v wy <sup>2</sup> f.....	168	101	98	81	127	119	104	104	55	34	46	46	53	47	23	25	47	35	58	62	16	12	795	666	1461
poi.....	13	21	22	8	21	13	15	7	7	8	3	4	10	7	5	4	7	3	10	4	4	4	120	83	203
ct <sup>6</sup> v wy <sup>2</sup> f.....	20	12	22	13	16	15	9	12	8	5	11	8	12	5	6	3	8	6	8	3	0	2	120	84	201
f.....	12	7	17	6	14	11	7	7	0	5	8	6	4	3	3	3	8	7	4	5	1	1	78	61	139
ct <sup>6</sup> v	13	11	14	5	9	7	11	9	5	5	1	6	7	2	4	2	1	2	6	5	2	1	73	55	128
wy <sup>2</sup> f.....	21	20	14	19	21	29	19	31	17	7	3	7	10	16	5	4	12	15	11	4	2	2	135	151	289
ct <sup>6</sup>	20	14	13	6	15	6	24	7	4	8	8	8	15	9	4	7	10	2	16	1	1	1	130	68	198
v wy <sup>2</sup> f.....	2	0	5	0	2	2	1	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	13	2	15
f.....	1	1	2	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	1	0	1	6	4	10
v wy <sup>2</sup>	..	..	1	0	..	..	1	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	3	2	5
ct <sup>6</sup> wy <sup>2</sup> f.....	..	..	..	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	0
v	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	0
ct <sup>6</sup> v	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	1	1
ct <sup>6</sup> v	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1	2	3
wy <sup>2</sup>	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	0
ct <sup>6</sup> wy <sup>2</sup>	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	0
ct <sup>6</sup>	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	0
v	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	0	0
347	208	291	192	320	272	253	219	132	98	112	111	156	110	68	54	126	93	135	104	26	26	1975	1547	3422	

TABLE 31  
 $X^4 \times 8V_T^{pol h} \times X$

Class	Number/broods																Total				
	140/4		150/3		121/3		122/2		119/2		M 2/2		M 5/3		m 0/3		M 7/2		♂	♀	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂			
ct <sup>6</sup> v wy <sup>2</sup> f.....	89	60	76	68	111	70	63	45	57	52	61	32	40	23	94	67	91	71	682	488	1170
poi.....	152	138	159	113	155	131	68	83	71	79	107	91	122	104	151	165	93	97	1081	1001	2082
ct <sup>6</sup> v wy <sup>2</sup> .....	19	13	16	17	26	18	16	16	19	9	18	17	9	8	28	25	17	14	168	137	305
f.....	21	14	24	20	25	13	15	17	13	11	8	13	16	16	25	19	22	12	170	135	305
ct <sup>6</sup> v.....	15	16	11	15	16	17	6	9	3	5	13	8	4	13	27	19	11	17	109	119	228
wy <sup>2</sup> f.....	21	10	9	8	13	9	8	7	6	3	13	6	7	3	18	11	8	6	103	63	166
.....	21	15	12	16	28	22	13	11	10	14	18	12	12	21	32	18	19	17	165	116	311
v wy <sup>2</sup> f.....	37	18	25	12	29	17	8	11	16	6	17	4	18	8	39	9	26	12	215	97	312
ct <sup>6</sup> f.....	0	1	0	0	3	5	...	3	2	3	0	0	0	0	7	1	5	5	17	18	35
v wy <sup>2</sup> .....	1	1	3	5	4	0	...	...	2	1	1	3	2	1	4	2	1	4	18	17	35
ct <sup>6</sup> wy <sup>2</sup> f.....	0	0	0	0	0	1	...	...	...	...	1	...	...	1	...	...	...	...	1	1	2
v.....	2	1	1	0	0	...	...	...	...	...	...	...	...	1	...	...	...	1	3	3	6
ct <sup>6</sup> v.....	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	1	...	...	0	1	1
wy <sup>2</sup> .....	0	1	0	0	1	...	...	...	...	1	...	...	...	...	...	...	1	1	2	3	5
ct <sup>6</sup> wy <sup>2</sup> .....	0	0	0	0	0	...	...	...	...	1	...	1	...	...	...	...	...	...	0	2	2
v f.....	0	0	0	0	0	...	...	...	...	...	...	...	...	...	1	...	...	...	1	0	1
	378	288	336	274	411	303	197	203	199	184	257	187	230	198	429	337	297	257	2755	2231	66

subsequent tests. It ought to be added that thus far no deficiencies at known loci have been found genetically in pointed, though such are known in the original px bl stock (see below); all available mutants between y and v were checked.

With these assumptions, it is to be expected that backcrosses with marked X chromosomes would give the following results: If both the second- and third-chromosome translocations are present in the autosomes of the pointed parents,  $F_1$  females may be heterozygous for one or both of the autosomal insertions, or they may be normal if the pointed parent was heterozygous; the mother of the backcross will be heterozygous for the marked and pointed X. The X might be deficient for one or the other or both deficiencies. The expectation of lethal classes in the males of the backcross  $poi/X\ ple \times X\ ple$  is that, in the absence of the transpositions in the first chromosome,  $\frac{1}{2}$  of the poi males will be missing in the plus class if one of the translocations is present, and  $\frac{3}{4}$  if both are present. The same is true for the cross-

TABLE 32  
CROSSOVER  $\bar{V}$  VALUES

	$poi \times v\ bl$	$poi$	Standard
ct-v.....	$12.2 \pm 0.0056$	$14.2 \pm 0.0050$	13
v-wy.....	$8.1 \pm 0.0047$	$8.3 \pm 0.0039$	8.9
wy-f.....	$12.7 \pm 0.0057$	$13.9 \pm 0.0049$	14.8

over classes with the left end from poi. In the presence of the transposition, all the noncrossover pointed males are viable, but crossover classes in which the transposition has been removed by crossing over from the section containing the deficiencies show the possible ratios listed in table 29. (Half the males are always free of the deficiency and we assume that the very small duplications are viable.)

Tables 30-34 contain data on such a backcross, separately for the two pointed alleles. The marker stock was  $X^+ = ct^+ v\ wy^+ f$ , and the poi X was introduced by the father. As the left end was not marked, the noncrossover class ct-f contains also some single crossovers left of ct, and as the deficiencies are located near the svr region, about 17 per cent of male lethals are added to the noncrossover group. But in the other classes containing cut the error is negligible, because only double and multiple crossovers are involved. We did not tabulate here the individual broods, but added sister broods from the same  $F_1$  cross ( $152/3$  means 3 sister pair crosses from  $F_1$  no. 152, all one-pair crosses). As table 32 shows, the crossover values are normal. In table 33 the sex ratios have been calculated for comparison with the expectations in table 29.

Keeping in mind the facts just pointed out, the summed-up results in all these tables show with perfect clarity that in the class v wy f about  $\frac{1}{2}$  of the males are missing, whereas the decisive noncrossover pointed class has an almost normal sex ratio. This latter fact excludes the presence of the 1-2 or 1-3 or both translocations as such, which would require a 1.33 or 1.6 sex ratio in the poi class. Therefore the lethal condition present in the v wy f class cannot indicate the locus of one of those deficiencies. It must mean that in addition to one or both translocations a duplicating transposition within the X chromosome must be present, inserted between v and wy. As the class wy f has also a high ratio, average 1.5, we must assume

TABLE 33  
SEX RATIOS IN THE BACKCROSSES ( $X^4 \times \text{poi}$ )  $\times$   $X^4$

Class	svr <sup>poi</sup>	svr <sup>poi h</sup>	Both	Reciprocal classes, ratio normal : deficient		
				poi	poi h	Both
poi.....	1.18	1.08	1.12	1.26	1.18	1.21
ct v wy f.....	1.37	1.4	1.38			
ct v wy .....	1.44	1.23	1.31	1.44	1.24	1.32
f.....	1.44	1.26	1.32			
ct v .....	1.28	.9	1.06	1.3	1.16	1.22
wy f.....	1.31	1.63	1.5			
ct .....	.88	1.13	1.0	1.19	1.56	1.39
v wy f.....	1.91	2.22	2.1			
ct f.....	6.5	.9	1.5	3.17	1.0	1.32
v wy .....	1.5	1.1	1.13			
All.....	1.23	1.28	1.25			

TABLE 34

RATIOS ♀ : ♂ IN RECIPROCAL CROSSOVER CLASSES OF FORTY INDIVIDUAL BROODS FROM WHICH TABLES 30-33 WERE CONDENSED. RATIOS ARE UPPER LIMITS OF CLASSES OF .5 UNITS  
(Frequencies of ratios n ♀ : 1 ♂)

C.o. class	Ratio n ♀ : 1 ♂															
	All ♂	0.4	0.9	1.4	1.9	2.4	2.9	3.4	3.9	4.4	4.9	5.4	5.9	6.4	6.9	7.4
ct v wy .....	1	3	8	10	6	3	5	3	..	1	..	..	..	..	..	..
f.....	1	..	10	15	3	5	2	3	..	1	..	..	..	..	..	..
ct v .....	2	6	6	17	6	..	..	1	..	..	..	..	1	..	..	..
wy f.....	..	2	2	13	4	12	..	1	2	1	..	1	..	..	..	..
ct .....	1	3	14	13	3	1	5	..	..	..	..	..	..	..	..	..
v wy f.....	..	2	4	9	4	4	2	..	2	2	..	1	..	2 <sup>a</sup>	..	1
+ poi....	..	1	11	23	3	..	..	..	..	..	..	..	..	..	..	..

C.o. class	Ratio n ♀ : 1 ♂															
	7.9	8.4	8.9	9.4	9.9	10.4	10.9	11.4	11.9	12.4	12.9	13.4	14	15	16	All ♀
ct v wy .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
f.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
ct v .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
wy f.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
ct .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
v wy f.....	..	..	..	1	..	2	..	..	..	..	..	..	1	..	2	..
+ poi....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

<sup>a</sup> All above 6 : 1 have only 1 ♂ each = 94 ♀♀ ♂.

that the locus of the transposition is about midway between vermilion and wavy. The relatively high sex ratio in the noncrossover  $ct\ v\ wy\ f$  class is, as already stated, the result of crossing over left of  $ct$ , which adds the deficiency near  $poi$  to this non-crossover class. Also, males with many recessive markers are less viable than females.

In the  $v\ wy\ f$  class the sex ratio is 2:1, and for both reciprocal classes it is 4:3. Looking at the table of expectations, we realize that this average result (after cross-over elimination of the transposition) does not indicate which of the possible combinations were realized in the 40 individual broods from which the tables were derived, i.e., whether one or both transpositions were present. All it tells us with certainty is that not all broods could have been of the types which require a sex ratio of 4:1 or 1:0 in the decisive crossover class. Thus, it is important to check upon the individual broods which had an average size of 118 females 94 males. The individual crossover classes in single broods are not large, which involves a considerably higher error. But actually the crossover classes  $ct-v\ wy\ f$  contain a larger number of individuals than the classes  $ct\ v\ wy-f$ , and therefore a smaller error is expected, which makes comparison of the two more reliable. Table 34 is a frequency table for the individual ratios in the crossover classes. It shows at sight that the classes  $ct\ v\ wy, f, ct\ v$ , and  $ct$  have a similar range of variation of the ratios, whereas  $wy\ f$  and  $v\ wy\ f$  are different. These classes contain high ratios which obviously represent the expected 1:0 ratios with a few males left, which may be the result of double crossover between  $poi$  and the deficiencies. The other ratios may include the normals, 2:1 and 4:1 (see table 29). This table, then, may be interpreted as showing: (1) that likewise in individual broods the transposition is never missing, since 2:1 and 4:1 ratios are absent in the  $poi$  class; (2) that in a number of broods (left end of the  $v\ wy\ f$  series) possibly no translocation is present, at least for the point near  $svr$  which is transposed to  $v-wy$ ; (3) that in a number of broods one translocation is present, covered by a duplication in the  $v-wy$  region (ratio 2:1 of the  $v\ wy\ f$  series); (4) that in a number of cases both translocations are present and, as it seems, both left loci are transposed to the right end, one in the  $v-wy$  region, the other possibly to the right of  $wy$  (the classes around 4:1 in all c.o. classes with the right end); (5) that in ten cases the almost complete absence of males indicates the presence of homozygous autosomes without insertion (see table 29).

It may finally be added that sister  $R\ F_2$  broods were also checked with respect to whether such sibs showed similar ratios in the individual broods. Looking at the ratios in the  $v\ wy\ f$  class, we found 8 such sibs of 2 or 3 broods, each, all of which had low ratios around 2:1 or 4:1.

The foregoing analysis was made with crosses in which the  $poi\ X$  was introduced by the father of  $F_1$ . As we found that females may have differently constituted  $X$  chromosomes (see the  $y$  crosses), a similar series was made by introducing the  $poi\ X$  via the mother, i.e.,  $(poi \times X') \times X'$ . Tables 35-39 show the results. Table 35 contains the summarized results for 25 broods each for  $poi$  and  $poi\ h$ , including the sex ratios of the larger classes; table 36 contains the crossover values; and tables 37-40 contain special data for the analysis of individual broods.

We notice first that the noncrossover class + ( $poi$ ) is practically equal for both alleles in both sexes. The  $X'$  noncrossover class is four times as large in  $poi\ h$  as in  $poi$ . This class with 7 recessives is, of course, very little viable. But this inviability

TABLE 35  
CROSSING OVER IN THE *poi* × *X*<sup>1</sup> CROSSES

Class		<i>poi</i>		<i>poi h</i>		Sex ratio	
		♀	♂	♀	♂	<i>poi</i>	<i>poi h</i>
svr (= <i>poi</i> = <i>svr</i> <sup>pos</sup> )		751	568	767	566	1.32	1.36
Single	y ec ct v wy f car	18	16	78	65	1.12	1.20
	y svr ec ct v wy f car	26	40	9	14	0.65	0.64
	y ec svr ec ct v wy f car	5	1	6	1	....	....
	y svr ec ct v wy f car	22	38	63	56	0.58	1.12
	y svr ct v wy f car	10	7	59	11	1.43	5.36
	y ec ct svr v wy f car	17	21	41	68	0.81	0.60
	y svr v wy f car	30	7	96	31	4.29	3.10
	y ec ct v svr wy f car	10	14	23	21	0.71	1.10
	y svr wy f car	72	8	53	18	9.00	2.94
	y ec ct v svr f car	6	4	22	19	1.50	1.16
	y svr f car	95	29	77	23	3.28	3.35
	y ec ct v wy f	18	13	22	10	1.38	2.20
	y svr car	62	36	54	34	1.72	1.59
	y car	3	1	3	..	....	....
	y f car	4	2	3	1	....	....
Double	svr ec ct v wy	2	..	3	..	....	....
	svr ec ct v	..	..	3	..	....	....
	y v wy f car	3	..	1	..	....	....
	y ec car	3	1	6	8	....	....
	svr ct v wy f	1	1	5	1	....	....
	y ec f car	1	1	11	13	....	0.85
	svr ct v wy	5	3	22	7	....	3.14
	y ec wy f car	..	..	1	1	....	....
	svr ct v	3	2	13	5	....	2.60
	y ec ct car	..	..	3	3	....	....
	svr v wy f	2	1	9	1	....	9.0
	y ec ct f car	..	..	3	2	....	....
	svr v wy	12	5	28	12	2.40	2.33
	y ec ct wy f car	1	1	2	..	....	....
	svr v	..	..	..	1	....	....
Triple	y ec ct v car	3	..	2	1	....	....
	y ec ct v f car	1	..	5	..	....	....
	svr ec ct car	..	..	1	..	....	....
Triple	y ec ct wy	..	..	1	..	....	....
	y ec v	..	..	1	..	....	....
Sa.....		1186	820	1496	993	1.45	1.51

is increased in *poi* (and, as we shall later see, also in *px bl*), showing that *poi* contains other debilitating features in the autosomes enhancing this inviability. Whatever differences we find thus between *poi* and *poi h* aside from this inviability are based upon different genetical features.

A look at the crossover values in table 36 shows at once that there is a considerable difference between the two *svr* alleles. In *poi* there is a large reduction of crossing



over from yellow to vermilion, aside from the sexual differences. A look at tables 35 and 37 shows one of the reasons for this difference, namely, the reduction of double crossing over in poi as compared to poi h, in addition to that of single cross-

TABLE 36  
CROSSOVER VALUES (poi AND poi h  $\times$  X')  $\times$  X' = y ec ct<sup>6</sup> v wy<sup>2</sup> f car

Segment	poi		poi h		Stand.
	♀	♂	♀	♂	
y-ec.....	3.6	5.4	1.9	1.6	5.5
ec-ct.....	3.8	6.5	12.1	10.3	14.5
ct-v.....	5.5	4.3	12.2	10.0	13.0
v-wy.....	7.6	3.0	7.6	4.7	8.9
wy-f.....	10.6	5.4	12.4	7.7	14.8
f-car.....	7.8	6.5	7.0	5.8	5.8
svr-ec.....	....	0.23	....	0.2	5.4

TABLE 37  
SINGLE AND DOUBLE CROSSOVERS AS PERCENTAGES OF THE NONCROSSOVER poi CLASSES

Interval	Single crossover				Double crossover			
	poi		poi h		poi		poi h	
	♀	♂	♀	♂	♀	♂	♀	♂
y-ec.....	4.1	7.2	1.9	2.6	1.6	0.5	1.8	0.2
ec-ct.....	4.3	8.0	17.2	12.4	1.7	1.4	7.7	6.4
ct-v.....	6.3	6.7	17.2	17.5	2.4	1.2	6.0	3.5
v-wy.....	10.9	3.9	10.0	6.9	1.1	0.5	3.7	1.4
wy-f.....	13.5	5.8	12.9	7.4	3.3	1.9	10.0	6.4
f-car.....	10.7	8.5	10.0	7.8	1.6	0.7	3.8	2.5
Totals.....	49.8	40.1	69.2	54.6	11.7	6.2	33.0	20.4

TABLE 38  
SEX RATIOS OF NONCROSSOVER poi CLASSES

	Ratio											
	Below 1	-1.3	-1.6	-1.9	-2.2	-2.5	-2.8	-3.1	-3.4	-3.7	-4.4	45:1
Frequency poi h.....	6	8	2	1	2	1	1	1	..	..	2	1
Frequency poi.....	5	9	4	3	..	1	1	..	..	..	1	..

ing over. In view of the identical sizes of the + poi classes, these values ought to parallel each other if the X chromosomes are constituted identically. In table 37, therefore, the individual single and double crossover individuals have been calculated as percentages of the noncrossover poi class, both for poi and poi h, and for all intervals. A comparison with the crossover table 36 shows: (1) Single crossing over is reduced considerably in the y-ec interval of poi h, but not of poi; double cross-

over breaks are nearly similar, thus showing fewer double crossovers in poi. (2) In the interval ee-ct crossing over is about normal in poi h but much reduced in poi; table 37 shows that both single and double crossovers share in this reduction. (3) In the interval ct-v, again, poi h shows almost normal crossover values, and poi much

TABLE 39

SEX RATIOS IN SINGLE CROSSOVER CLASSES WITH MISSING MALES, CORRELATED TO THE SEX RATIO OF THE NONCROSSOVER poi CLASS

Single crossover class	poi Sex ratios of the noncrossover poi class								
	Below 1.3 (14)			1.3-1.8 (7)			Above 1.8 (3)		
	♀	♂	♀ : ♂	♀	♂	♀ : ♂	♀	♂	♀ : ♂
v wy f car.....	12	2	0.6	12	3	4	4	3	1.33
wy f car.....	43	8	5.4	23	..	∞	6	..	∞
f car.....	64	21	3.0	26	3	8.7	5	7	0.7
car.....	34	17	2.0	25	12	2.1	3	3	1.0

Second column, test for transposition ratio 4:1:  $\chi^2=5.0$ ,  $P<0.05$ ; ratio 10.1:1, P.E.=2.2, Dev. exp. ratio 3.2.

Total: all single crossovers with left end not from poi; poi h=180 ♀ 188 ♂, poi=99 ♀ 132 ♂. The same with left end from poi; poi h=345 ♀ 128 ♂, poi=274 ♀ 88 ♂. The only large double crossover class v wy has, all together, 12 ♀ 5 ♂ for poi, 28 ♀ 17 ♂ for poi h.

TABLE 40

(Continuing from table 39)

SEX RATIOS IN SINGLE CROSSOVER CLASSES WITH MISSING MALES, CORRELATED TO THE SEX RATIO OF THE NONCROSSOVER poi CLASS

Single crossover class	poi h Sex ratios of the noncrossover poi class											
	Below 1.3 (14)			1.3-1.9 (3)			1.9-3.1 (5)			Around 4 (2)		
	♀	♂	♀ : ♂	♀	♂	♀ : ♂	♀	♂	♀ : ♂	♀	♂	♀ : ♂
v wy f car.....	59	22	2.7	3	..	∞	7	3	2.3	6	..	∞
wy f car.....	33	16	2.1	3	..	∞	4	1	4.0	4	..	∞
f car.....	49	18	2.7	7	1	7.0	4	2	2.0	9	1	9
car.....	41	22	1.9	2	3	0.7	4	5	0.8	2	1	2

Fourth column:  $\chi^2=1.8$ ,  $P>0.10$ ; ratio 10.5:1, P.E.=1.3, Dev./P.E.=2.3.

reduced ones; again, the relative share of single and double crossovers is not very different in both cases. (4) In the interval v-wy, crossing over is normal in the females and reduced in the males of both alleles. There are relatively more double crossovers in poi h. (5) In the interval wy-f, again, crossover values are about normal in females and low in males. In both sexes more double crossovers contribute to the results in poi h. (6) In the interval wy-car the same is true. (7) The sum total in table 37 shows clearly that altogether single crossover is about 25 per cent less frequent in both sexes of poi as compared with poi h. Double crossing over is about three times as frequent in poi h as in poi, so that the scarcity of double crossing

over is mainly responsible for the low crossover values in the female and male classes of poi visible in table 36. Thus we see disturbances of crossing over:

for both sexes in ec-v of poi, not poi h  
 for males only in wy-f of poi and poi h  
 for both sexes in y-ec of poi h only  
 for none in f-car of both alleles.

Of these disturbances those located in the v-f interval act differentially in poi by the reduction of survivors from double crossing over. Those in the interval ec-v act in poi differentially by reducing both single and double crossover. Thus we expect in the interval v-f a disturbance affecting the males of both alleles, and a difference between them affecting double crossover survival in both sexes. In the interval ec-v only poi contains a disturbance affecting both single and double crossing over. Also, poi h had another disturbance affecting crossing over in the y-ec interval.

In table 36 the svr-ec crossover value is also found, taking into account that svr cannot be distinguished in the presence of yellow. The percentage is very small. We remember (see p. 299) that in addition to the well-known crossover irregularities at the left end of the X chromosome the values calculated for the poi stock are rather erratic.

Returning now to the crossing-over data summarized in table 35, we find that the sex ratios are about normal, even sometimes in favor of the males in the noncrossover classes and in all crossover classes not containing the left end of the original poi chromosome, but that they are high or very high in the reciprocal classes. Simple sex-linked lethals are excluded, and we must again think of translocations and transpositions. For simple translocations alone from X to an autosome producing  $\frac{1}{2}$  male lethal deficiencies in each case the expectations have already been tabulated (table 29), namely, 2 : 1 and 4 : 1, respectively, in the poi class and the proper crossover classes. Clearly, the poi class with large numbers does not show this ratio. But neither does it show a 1 : 1 ratio. This suggests that the 25 resp. 24 individual broods of which the poi and poi h crosses are composed may be different, i.e., the original-stock females may have been heterozygous with respect to the features studied here. Therefore we tabulate the frequencies of sex ratios found in the noncrossover poi class in table 36. The table indicates that the poi class contains a mixture of sex ratios, with a majority of cases 1 : 1, and a minority of 2 : 1, and 4 : 1 in both cases. There is one additional brood in poi h crosses with a single poi male among 46. This completely abnormal brood contains only 5 males altogether, with 67 females. With the exception of 2 females in the y ec class, all remaining 21 crossover females are found in the crossover classes containing the left end of the poi chromosome. The most probable explanation is that the poi X chromosome contained a lethal mutant (not present otherwise) and that another lethal mutant had arisen in the X of the tester stock, thus also permitting female lethal combinations. As these were present in all classes to the right of ec, i.e., absent in the y-ec regions, whereas the absence of males characterized all classes with the same region from poi, the new lethal in the tester chromosome must be located to the right of ec and the one in the poi chromosome to the left of this point. As one male poi survived, which must have been owing to crossover between svr and the lethal, the latter would be located even to the left of yellow. The four other surviving males (two each) were found in the y ec ct and v wy f car classes, which would suggest that the new lethal in the tester

chromosome was located between *ct* and *v*. But this would require also double crossing over between *svr* and the lethal. The explanation is unsatisfactory and the case has not been followed up.

To return to the other members of this series: If the ratios of 2 : 1 and 4 : 1 in the *poi* classes are significant, the two translocations studied above must have been present in some of the crosses, whereas the majority did not contain them. If in the latter group, however, typical aberrant sex ratios are found in definite crossover classes, we suspect again the transposition in the *X* chromosome which we had to assume before. Therefore (table 39, 40) we separated the data for the single crossover values in accordance with the ratios found in the noncrossover *poi* class. (Only a few double crossover classes have sufficient numbers.)

In the table the first columns for both alleles contain what probably is a 1 : 1 sex ratio for the noncrossover *poi* classes, which means either no translocations and transpositions, or deficiencies by translocation covered by transpositions, or only transpositions. In the first case, the crossover sex ratios should be normal. In the second case, they should be normal for the classes containing the right end of the *poi* chromosome (supposed to contain the transpositions) but deficient with only the left end of the *poi X*, namely, 2 : 1 if only one translocation-transposition is involved, 4 : 1 if both are present. In the third case, all males with only the left end from *poi* should be missing. The data for *poi* (first column) suggest clearly that both translocations and transpositions are present, and, further, that one of the transpositions is located in the *v-wy* region and the other right of the *ca* region. The abnormal ratios in the class which also contains *ct* (not found in table 39, 40) locate the deficiencies again in the left end.

The column for *poi h* shows different results. Here all crossover classes with only the left end of the *poi X* have a ratio around 2 : 1. This shows that only one of the translocations is present, covered by the transposition in the *ca* region.

In the event of high ratios for the noncrossover *poi* class (2 : 1, 4 : 1), one or both translocations must be present. In the absence of transpositions, all classes with the left end from *poi* must show the same ratios as the noncrossover class. In the presence of transpositions, the expectations are the same as before, as is exemplified in the classes with normal ratios for *poi*. Unfortunately, the numbers are rather small for these rarer groups and do not permit of a decision. The two groups for a probable 2 : 1 ratio in *poi*, i.e., either one translocation alone or both plus one transposition, seem rather alike, and, if added (columns 3 in *poi* and *poi h*), may represent a 4 : 1 ratio for the first two crossover classes and a 2 : 1 ratio for the others. This would mean two translocations with one transposition in the *v-wy* region. There are, further, two columns with *poi* ratios between 1 : 1 and 2 : 1 (second columns). The ten broods therein may belong partly to the one or partly to the other class. In the *poi h* group it seems probable that they belong mostly to the 2 : 1 class, though the numbers are too small for a decision. In the column for *poi* the first and the last crossover class agree with the results of the first column. But here we find no males in the *wy f* car class, and only a few in the *f* car class, a result which we would expect only if a transposition and no translocation were present, the transposition being located between *wy* and *f* (see statistical test in tables 39-40).

There are, finally, the two *poi h* broods with a 4 : 1 ratio for the *poi* class. Here two translocations and no transpositions ought to be present, and there should there-

fore be a 4:1 ratio in all cross over classes of the table. The small numbers may permit this explanation. Altogether we may conclude that the poi stocks may contain translocations from the left end of the first chromosome into the second, the

TABLE 41  
svr<sup>poi h</sup>/ec, a px sp/+ × ec, a px sp/a px sp

	♀	♂	Normal expectation	
			♀	♂
+	120	5	1	C.o. poi-ec
poi	..	127	..	1
ec	96	99	1	1
a px sp	78	5	1	C.o. poi-ec
ec, a px sp	116	71	1	1
poi, a px (sp)	..	31	..	1
poi ec	..	..	..	C.o. poi-ec
poi ec, a px sp	..	..	..	C.o. poi-ec
Σ	410	338	No crossover a-sp found	

third, or both, and in addition duplicating transpositions of the same left-end loci, one into the v wy region and another beyond car, and also that the possible viable combinations of all these may be present.

TABLE 42  
svr<sup>poi h</sup> +/+ v, a px sp/+ × v/ a px sp/a px sp

Class	♀	♂	Normal expectation	
			♀	♂
+	103	14	1	½ c.o. poi-v
poi	..	48	..	1
v	76	71	1	1
a px sp	59	25	1	¼ c.o. poi-v
v, a px sp	74	42	1	1
poi, a px (sp)	..	33	..	1
v, a px	10	19	..	½ c.o. px-sp
v, sp	9	2	..	½ c.o. px-sp
poi v	..	8	..	¼ c.o. poi-v
poi v, a px (sp)	..	7	..	¼ c.o. poi-v
	331	269		

It is rather unfortunate that tests made with a large number of mutant loci never revealed the presence of known loci in the deleted or translocated sections. Thus a more detailed analysis was made impossible. But a few tests were conducted with two marked chromosomes in which the decisive regions in the first, second, and third chromosome were marked, namely, by echinus (ec) near poi, near which the deficiencies by translocation are supposed to be located; vermilion (v), which is near the transpositions in the first chromosome; a px sp in the region of the second

chromosome insertion, simultaneously a safe mark for pointed because of speck suppression; ebony (e), not far from the region of insertion in the third chromosome. Tables 41-43 contain three such backcrosses with the expectations if everything were normal. The first backcross of  $svr^{poi\ h}/ec$ , a  $px\ sp/- \times ec$ , a  $px\ sp/a\ px\ sp$  shows the expected number of females except that the class a  $px\ sp$  is rather small. This class has the composition a  $px\ sp/a\ px\ sp$ ,  $poi\ +/-\ ec$ . If the smallness of the class is significant—it actually recurs in cross no. 2,—and if it is not based on lower viability of the marked chromosomes, which is improbable in view of the perfect class  $ec$ , a  $px\ sp$ , crossing over in the first chromosome of the mother must be responsible. As  $ec$  is very near  $poi$ , many crossovers to the right of  $ec$  are expected

TABLE 43  
 $svr^{poi\ h} +/+ \ ec, +/e \times ec, e/e$

Class	♀	♂	Normal expectation	
			♀	♂
+	87	5	1	C.o. $poi-ec$
$ec$	86	76	1	1
$poi$	..	48	..	1
$e$	84	1	1	C.o. $poi-ec$
$ec, e$	70	73	1	1
$poi\ e$	..	29	..	1
$poi\ ec$	..	6	..	C.o. $poi-ec$
$poi\ ec, e$	..	..	..	C.o. $poi-ec$
	327	238		

which would produce backcross females without the transposition in the X chromosome, if present. These will be heterozygous both for the 1→2 and the 1→3 deficiency, which might impair their viability. But the decisive group are the males. The  $poi$  and  $ec$  males are present in the expected numbers. The class  $ec$ , a  $px\ sp$  males are just as deficient as the a  $px\ sp$  females. In this class it is possible that the duplication from 1→3 translocation and transposition is present in triplicate (via crossover beyond  $ec$ ), which might account for lower viability. The class  $poi$ , a  $px\ sp$  is only about one-fourth of the expected size. In the presence of the 1→2 translocation, all, or almost all, males ought to be missing. But if the transposition is also present in the  $poi$  chromosome, all  $poi$  males ought to survive except for crossing over separating the transposition from  $poi$ . This is clearly the case in the small  $poi$ , a  $px\ sp$  class. But the same ought to be true for the  $poi$  class, which, however, is quite normal. This shows that only the second-chromosome translocation (and deficiency near  $poi$ ) was present, and not the translocation to the third chromosome. A further check is furnished by the crossovers between  $poi$  and  $ec$  giving one-half plus, mostly with the transposition, if the deficiency by transposition is located near  $svr$ , and one-half  $poi\ ee$  without the transposition. The latter ought to be lethal with a  $px\ sp$  but for double crossovers. Actually, five crossover males each are found in the plus and a  $px\ sp$  classes, and none in the two  $poi\ ec$  classes. Thus the data fit our former analysis fairly well, indicating that by chance a  $poi$  chromosome with only the deficiency by translocation into the second chromosome was used.

In the second cross (table 42), in which *ec* is replaced by *v*, the females show approximately the same results as in no. 1. As vermilion is located almost 33 units to the right of pointed, considerable crossing over between the two is expected. In this case also some crossovers between a *px* and *sp* were found which had not appeared in the first cross. The percentages are near expectation for both. The summarized sex ratio shows at once that almost one-fourth of the males are missing. The majority of these are clearly contained in the *poi* and *poi*, a *px sp* classes. If we have our old translocations and transpositions, the *poi* class ought to be normal except for crossing over beyond *v*, between this and the transpositions, thus removing the transpositions from *poi*, i.e., making lethal all these crossovers combined with the unmarked third chromosomes which do not contain the translocation. The same is true also for the *poi*, a *px sp* combination where, in addition, the foreign second-chromosome combination (a *px sp*) is lethal when the proper transposition is removed by crossing over beyond *v*. Further, in the two small crossover classes, *poi v* one-half or all (with a *px sp*) ought to be missing because the transposition is absent, but for double crossovers between *v* and the transposition. Actually the numbers are much smaller than those for the reciprocal ++ classes. The fact that so many survivors are found in the *poi v*, a *px sp* class where only double crossovers can survive points to the transposition near *ca* as the one which is identical with the translocation into the second chromosome, the long intervals *poi - v - ca* permitting considerable double crossing over. There remains only the class *v*, a *px sp* (the small number of a *px sp* females has already been discussed with the former cross). Here a considerable number of males are missing. On the basis of our hypothesis these could be only double crossovers between *poi*-deficiencies-*v*, combining the deficiencies with vermilion without *poi*. On the whole, then the data confirm the interpretation.

The third cross (table 43) is parallel to the first but for the marking of the third chromosome. In the presence of both transpositions *poi* males ought to be normal. But all crossing over to the right of *ec* and to the left of the transposition will remove the transposition, whereas *poi* and the deficiencies remain, thus producing lethal *poi*. Actually, the *poi* class is far below expectation and still more so the class *poi*, *e*, where all the crossovers will die, whereas in the *poi* class only those with the homozygous second chromosome die. Among the crossovers *poi-ec* the *poi-ec*, *e* class must be lethal since the transposition is certainly absent, whereas in *poi ec* only half of the second-chromosome combinations will be lethal. Again the results confirm the interpretation.

*The F<sub>1</sub> ratios.*—This makes us return to the *F<sub>1</sub>* ratios reported above, namely, normal ratios in the crosses *N* × *poi* and *y* × *poi*, but variable abnormal ratios in the crosses *poi* × *N* (see table 12). In table 44 a number of possibilities for the constitution of the pointed female have been assembled, together with the resulting *F<sub>1</sub>* sex ratios. We did not assume homozygosity for the autosomal translocations, which, apparently are viable. They would lead to more normal ratios in the different classes, intermediate between those expected and normal. Homozygous deficiencies of the X chromosome are not expected to exist, though a case of complete absence of males occurred which might have been a case in point. The possibility of a single or double crossover between the loci of the two transpositions has been neglected. Such crossovers would further modify some ratios. Altogether, we expect to en-

counter most frequently ratios between 1 and 2, all over the range of this interval, further, ratios of 4:1 and many : 1—in agreement with the results in table 12.

Thus we may say that in a general way the experiments give consistent results, though there are incongruities in detail which indicate that the situation might be still more complicated. As the rearrangements are extremely small (see next section) and never could be shown to contain known loci, a further analysis does not look promising, and it would hardly be important for the problem which made us look into such minute details.

TABLE 44

SOME OF THE SEX RATIOS OBTAINABLE IN  $F_1$  *poi* × *N* WITH DIFFERENT CONSTITUTIONS OF THE AUTOSOMES AND SEX CHROMOSOMES IN REGARD TO THE NONRECIPROCAL TRANSLOCATIONS 1→2 AND 1→3 (MARKED T), THE CORRESPONDING DEFICIENCIES IN THE LEFT END OF X MARKED Df 1-2 AND Df 1-3, AND THE LEFT-RIGHT TRANSPOSITIONS IN THE X CHROMOSOME CORRESPONDING TO THE TRANSLOCATIONS, MARKED Tr 2 AND 3. CROSSING OVER BETWEEN THESE TWO TRANSPOSITIONS IS DISREGARDED.

2d chromosomes of mother	3d chromosomes of mother	One X chromosome of mother	Other X chromosome of mother	Sex ratio $F_1$ ♀ : ♂
+/T	+/+	Df 1-2	+	1.33
+/T	+/+	Df 1-2	+	1.60
		Df 1-3		
+/T	+/+	Df 1-3	+	2.00
+/T	+/+	Df 1-2	Df 1-3	4.00
+/T	+/T	Df 1-2	Df 1-3	2.00
+/T	+/T	Df 1-2	+	4.00
		Df 1-3		
+/T	+/+	Df 1-2, 1-3	+	1 + $\frac{1}{4}$ c.o. Df-Tr
		Tr 2, 3		
+/T	+/T	Df 1-2, 1-3	+	1 + $\frac{1}{8}$ c.o. Df-Tr
		Tr 2, 3		
+/T	+/T	Df 1-2, 1-3	Df 1-2	1.23
		Tr 2, 3	Df 1-3	
+/+	+/+	Df 1-2	Tr 2, 3	2 — c.o. Df-Tr
		Df 1-3		
+/+	+/+	Df 1-2	+	2
		Df 1-3		
+/+	+/+	Df 1-2	Df 1-3	No ♂♂ except $\frac{1}{2}$ c.o. Df 2-3

*The salivary-gland chromosomes of  $svr^{po}$  and  $svr^{po+}$ .*—The cytological features of the  $svr^{po}$  locus or region itself will be presented below, together with those of further alleles, because they cannot be described simply. Suffice it to say that the locus of  $svr$ , i.e., left of the break of a known deficiency, is normal. Here we present only the findings apart from the pointed locus or segment itself. The experimental results have already indicated that only very small sections could be involved, and further, that the individuals were not supposed to be alike in regard to the small deletions, translocations, and transpositions. Therefore, two types of checks were made. First, slides used for the study of the pointed locus itself were generally inspected for other regions. Secondly, a systematic check of a series of crosses and one-gland slides was made in the following way.<sup>5</sup>

<sup>5</sup> This check was made by Mr. Masuo Kodani under the supervision of the author. Later, many points were rechecked in the same slides by Miss Aloha Hannah, always with positive results.



A number of female as well as male *poi* and *poi h* were crossed to Oregon stock which had been checked for normality in the respective regions. From each cross a number of one-gland (one-cross) slides were made, and they were checked individually. In addition, a number of homozygous *poi* slides were made and checked for presence of the heterozygous condition of the respective rearrangements only. Thus a picture of the distribution of the rearrangements in the mutants was obtained. But only the positive results are important, since negative ones need not always mean absence when single or few bands, sometimes in difficult or unsuspected regions, are involved. The diagnosis given was derived from a comparison with the normal stock as well as with the published standard maps and was accepted only when the senior and junior authors gave identical interpretations. There is no doubt that the rearrangements to be described are present; but many others might have been found if the genetically unsuspected regions had been checked as thoroughly for single bands as were the regions indicated by the experimental results. The findings therefore represent minimum results. Altogether, 20 different small rearrangements were found with certainty, some of which, however, have a special meaning (see below). Tables 45 and 46 contain the condensed statistical data. The tables show that 12 out of 20 small rearrangements are found both in *svr<sup>poi</sup>* and *svr<sup>poi h</sup>*, which are of completely different ancestry, and further, that 4 in each were not found in this series in the other allele. Only a few of them have been found in all crosses, as the last column indicates. The rearrangements involve mostly one or two bands, maximum four. As was said before, only reliable cases were tabulated, and, with such small sections involved, this excludes a perfect record. But one might safely say that the actual occurrences will be roughly proportional to the findings. Regions with crowded small bands which are clearly visible only in case of perfect stretching may, however, contain too small a number of positive cases, in comparison with more favorable regions.

In the tables, the first column contains a description of the rearrangement. The next three columns indicate the number of pair matings for three combinations, and the fourth column, the number of one-gland—one-larva—slides made for all crosses; actually, in table 45, 15 slides *poi* × Ore, 70 Ore × *poi*, and 10 *poi* homozygous. The following columns indicate the number of glands in which the respective rearrangement was found, dubious cases being enclosed in parentheses. The last column contains information about the presence of the rearrangement in the 12 (in table 45) individual crosses. Table 46 is to be read in the same way. Furthermore, a column indicates where the individual occurrences are illustrated in plates 24–27.

There are in both tables a number of apparent rearrangements which we do not consider to be legitimate. All these are deletions and translocations of chromosome tips. These are numbers 1, 9, 10, 16 for *poi*, and 1, 9, 11, 15 for *poi h*. Their probable meaning has been discussed in a special paper (Goldschmidt and Kodani, 1943). An attempt was made to show that these are only pseudo-deficiencies and translocations, caused by artificial rupture of chromosome tips after attachment to each other.

In the X chromosomes of this series three deficiencies and two insertions were found: in *svr<sup>poi h</sup>*, deficiencies nos. 3 and 4, and insertions nos. 2 and 5; in *svr<sup>poi</sup>*, the same insertions (nos. 2 and 4), but only one deficiency. The no. 2 insertions were different in the two alleles, with only one band in *svr<sup>poi</sup>* and three in *svr<sup>poi h</sup>*. This latter insertion is located to the right of 3C7, i.e., between facet and echinus. A

different aspect of this insertion is presented in plate 28, fig. 5. As the table indicates, this insertion was found in almost every gland. For some time, it actually misled us into believing that this was a translocation of the speck locus and that pointed was this translocation; later, it turned out that pointed was a *svr* allele with the *sp* suppressor action found in other suppressors of the same region. As the genetical results made us expect a lethal deficiency in this general region (though not necessarily at this point), but no translocation or transposition, the section was studied over and over again; but there was no doubt that we saw an actual insertion. As has already been mentioned, this insertion may be considered as always present in both alleles, though its size is different in both. The insertion is not easily studied, because for an inexplicable reason the group of thick and thin bands of the *w-fa* region are invariably contracted into a thick chromatic mass (only in the *poi* slides, not in controls) in which the bands are hardly recognizable. The insertion which follows the *fa* band is usually included in this mass. As far as could be made out, there are three bands in *svr*<sup>poi</sup> and one in *svr*<sup>poi</sup>.

Looking over tables 45, 46, we realize that, with the lone exception of the pseudo-translocations of chromosome tips, this is the only rearrangement which is ubiquitous in both alleles; and we may add that this insertion is always absent in *px bl* (as well as Oregon), though many of the other rearrangements were also found in *px bl*. This insertion, then, must have played some role in the independent origin of the two pointed alleles! We did not succeed in finding out with certainty where it comes from. No synaptic association with another chromosome was observed, and no such association with another part of the X which could point to a transposition. Actually there is a deficiency, only in *poi h*, in section 4 of the X chromosome (no. 3, table 45) the three bands of which correspond to those of the insertion, and this locus is near 10 of the genetic map, i.e., between *ec* and *cv*. Is it possible that one of the two transpositions from left to right which the genetical data indicated is actually from right to left? The crossover experiments ought to give such information, but the facts could never be interpreted thus.

It ought to be repeated that the insertion is not easily studied. In all slides of the *poi* series, though not those of the controls, the group of conspicuous bands to the right of which the insertion is located (the white-roughest-facet group) tends to contract into an X-shaped mass of chromatin which can hardly be analyzed. The tiny inserted bands tend to disappear in this tangle. But when the *fa* band is clearly separated, the insertion also becomes visible between this and the broken band to the right, both of which are easy to recognize.

The only other chromosome abnormalities found in the X chromosome of both alleles is a deficiency (no. 4, table 45, for *poi h*) of two bands in section 9, to the left of vermilion. This is not expected on the basis of the genetical data, though some of them which have been interpreted differently might be connected with this deficiency, which was found in 7 out of 12 crosses. (We shall meet later with a vermilion deficiency in *px bl*). Plate 28, figure 7, shows this deficiency in a well-stretched and not synapsed chromosome section. But the main failure of the cytological check is that the transposition between vermilion and wavy indicated by the genetical tests was not regularly found. A one-band insertion at 12 E, i.e., in the proper region, was actually found (table 46, *poi h* no. 5, and table 46, *poi* no. 4) in both alleles, but only three times in one gland each of 3 out of 12 crosses. This is not satisfactory.

TABLE 45  
SALIVARIES OF SVT<sup>poi</sup>h

No.	Rearrangement	Number of bottles (one pair)			No. of slides	Number of slides positives <sup>a</sup>			Plate and fig.	Remarks
		poi X Ore	Ore X poi	poi homo		poi X Ore (15 slides)	Ore X poi (70 slides)	poi homo (10 slides)		
1	Df and T(X)1A1-4.....	3	7	2	95	6 ♀	36 ♀	.....	b	In all but 1 cross
2	T(X)3C.....	3	7	2	95	2 (8) ♀ 1	49 ♀	.....	25/2	In all crosses
3	Df(X)4C9 and 4D1, 2°.	3	7	2	95	.....	5 (1) ♀	.....	23/4	Only in 1 cross
4	Df(X)9A2, 3.....	3	7	2	95	5 ♀	.....	2 ♀	25/1, 2	In 3 out of 4 crosses
5	T(X)12E.....	3	7	2	95	.....	1 ♀	.....	25/9	1 cross
6	Df(2R)48C5 and D5°.	3	7	2	95	.....	10 ♀ ♂	.....	24/5	Only in 2 crosses
7	Df(2R)53D8°.	3	7	2	95	.....	4 ♀ ♂	.....	24/6	Only in 1 cross
8	Df(2R)58D5.....	3	7	2	95	.....	12 (6) ♀	.....	26/1	Only in 3 crosses
9	Df(2R)60F4, 5.....	3	7	2	95	5 (2) ♀ ♂	8 ♀ ♂	.....	b	4 out of 10 crosses
10	T(2L)24AB.....	3	7	2	95	.....	5 ♀	.....	24/4	Only 1 out of 7 crosses
11	T(3L)61A.....	3	7	2	95	2 (2) ♀ ♂	17 ♀ ♂	6 ♀	b	In 9 out of 12 crosses
12	Df(3L)61C8.....	3	7	2	95	.....	.....	(2) ♀ ♂	26/2	Only 1 cross
13	Df(3R)86C6, 7, 8.....	3	7	2	95	9 ♀ ♂	8 ♀ ♂	.....	26/7	In all crosses with ♀ poi; in 2 out of 7 recipr. crosses
14	Df(3R)84D6, 7.....	3	7	2	95	.....	.....	.....	26/4	4 Found only in crosses outside of this series
15	T(3R)100F.....	3	7	2	95	6 ♀ ♂	45 ♀ ♂	3 ♀ ♂	b	In all crosses, 3 times in all 10 slides
16	Df(3R)91C4, 5.....	3	7	2	95	.....	.....	.....	26/3	Found only in crosses outside of this series

<sup>a</sup> Numbers in parentheses = probable but not certain cases. ♀ ♂ = found in chromosome of female and male larvae.

<sup>b</sup> Not illustrated ones have been published already by Goldschmidt and Kodani.

<sup>c</sup> Found only in poi 1, not in poi.

TABLE 46  
SALIVARIES OF SVT-poi

No.	Rearrangement	Number of bottles (one pair)		No. of slides	Number of slides positive <sup>a</sup>			Plate and fig.	Remarks
		poi X Oro	Oro X poi		poi X Ore (20 slides) 5 ♀	Oro X poi (5 slides) ...	poi homo (15 slides) ...		
1	Df(X)1A1-4.....	4	1	3	40	...	...	b	All of one cross
2	T(X)3C (diff. from poi h, fewer bands).....	4	1	3	40	1 ♀	...	25/7	In all crosses
3	Df(X)9A2, 3.....	4	1	3	40	...	2 ♀	25/1, 2	In 4 out of 8 crosses
4	T(X)12E.....	4	1	3	40	...	...	25/9	In 2 out of 8 crosses
5	T(2L)24AB.....	4	1	3	40	2 ♀ ♂	3 ♀ ♂	24/4	In 5 out of 8 crosses
6	Df(2R)58D5.....	4	1	3	40	6 ♀ ♂	3 ♀ ♂	26/1	In 4 out of 8 crosses
7	Df(2R)58E.....	4	1	3	40	2 ♀	1 ♀	24/7	In 2 out of 8 crosses
8	Df(2R)60D5, 6.....	4	1	3	40	1 ♀	...	24/8	In 2 out of 8 crosses
9	Df(2R)60F4, 5.....	4	1	3	40	2 ♀	...	...	In 2 out of 8 crosses
10	T(3L)61A.....	4	1	3	40	2 ♀	...	...	In 2 out of 8 crosses
11	Df(3L)61C8.....	4	1	3	40	3 ♀ ♂	2 ♂	26/2	In 2 out of 8 crosses
12	T(3L)70BC and 71BC.....	4	1	3	40	...	3 ♀ ♂	....	In all homo poi
13	Df(3R)84D6, 7.....	4	1	3	40	7 ♀ ♂	8 ♀ ♂	26/4	In all crosses
14	Df(3R)86C6, 7, 8.....	4	1	3	40	7 ♀ ♂	8 ♀ ♂	26/7	Same as 13
15	Df(3R)91C4, 5.....	4	1	3	40	...	1 ♀	25/3	Only 1 homo poi
16	T(3R)100F.....	4	1	3	40	7 ♀ ♂	1 ♀	b	In 6 out of 8 crosses

<sup>a</sup> ♀ ♂ = found in chromosome of female and male larvae.  
<sup>b</sup> Not illustrated ones have been published already by Goldschmidt and Kodani.  
<sup>c</sup> Found only in poi, not in poi h.

The other transposition which ought to be located near the chromocenter was never encountered. But this, again, is a rather difficult region for very small disturbances.

In the second chromosome of  $svr^{po1}$ , 3 deficiencies were found in the right arm—leaving aside pseudo-deficiency no. 9,—but no insertion. The two infrequent deficiencies, nos. 6 and 7, are located in a region for which we have no genetical information. The third, no. 8, is the most frequent one and is present in both alleles in 7 out of 12 crosses, namely, the absence of band 58D5. This is the arc region. Since one of the alleles of the mutant broad angular is, as we shall see later, a deficiency of one (double?) band in this region, namely, 58D6, 7, and since the experiments indicated an insertion in this region, another investigation was made. Actually, a one-band insertion was found for  $svr^{po1}$ , a little to the left, namely, between 58D2 and 3 (pl. 28, fig. 6). In  $svr^{po1}$  an additional two-band deficiency, 60D5, 6, was found in a few cases, located to the right of balloon. It is clearly derived from  $px\ bl$ , and it will be discussed in the section on  $px\ bl$ . In both alleles, furthermore, a one-band translocation was not infrequent in the left arm of the second chromosome between 24A and B, a region not far from dumpy. The origin could not be found, nor any genetic manifestation.

The third chromosome furnished quite a number of small rearrangements. Aside from the pseudo deficiencies and translocations at the tip, T(3L)61A, T(3R)100F, we found in  $svr^{po1}$  one deficiency and one translocation in 3L (nos. 11, 12), the latter not being present in  $svr^{po1}$ . The deficiency was rather frequent, the translocation less so. The deficiency is located near the free end (one band only) and might be responsible for the effects which we found located near  $ru\ h$ . The translocation, which was found only in homozygous  $svr^{po1}$ , and here in all glands, is located near *Lyra* and *Dichaete*. As this is not far to the right of *h*, and as we found a powerful dominant enhancer of pointed in this region, we assume that this translocation represents the enhancer; enhancing actions of rearrangements are well known (see Goldschmidt and Gardner, 1942; Gardner, 1942). In 3R three deficiencies were found in both alleles. One of them was almost always present in both alleles, namely, Df(3R)86C6, 7, 8, which is a location between pink and curled. In some crosses with third-chromosome markers, not reported above, a lethal condition near *cu* seemed to exist, but the data were not unequivocal, and after some repetition with variable results we gave up further checks. The other, Df(3R)84D6, 7, was frequent in  $svr^{po1}$  and rare in the other allele. The location is to the left of pink, and no genetic effect was found. Another rare deficiency, Df(3R)91C4, 5, is located near *ebony*. The small translocation from X, which the genetic data required was found only once, namely, the insertion of one thick (double?) band in 91B (pl. 28, fig. 8).

Besides the disturbances found in this check series, a few more were observed which did not belong to this series. In the X chromosome a small Df(X)3D5—only one band—was found occasionally. The location is between *fa* and *ec*, both loci being known to offer abnormal genetic behavior. Further, in the region near the spindle fiber of 3R, where some small Df had been found, another was observed, Df(3R)82E6 or 7. A number of these and other rearrangements found outside the test series are shown in table 4 and 5 (see legend).

## b. THE MUTANT broad angular (bran)

In the general description of the first mutational changes, we mentioned a mutant broad angular (abbr.: bran) which appeared simultaneously with pointed and has reappeared many times since. We have already stated that the recombination of homozygous bran (i.e., broad rounded = broad angular, i.e., sometimes more rounded and sometimes more angular wings) with pointed homozygous or simplex produces a wing type described in my records as soft blistered. Some of the alleles of pointed can be best distinguished in this or a corresponding combination effect. Hence we shall first study bran, and afterward return to the other pointed alleles.

*Phenotype and localization.*—The original mutant showed considerably broadened, shortened, and rounded-off wings. In most of its later recurrences, however, the wings were not rounded but were more angular or squared at the tip. As extracted bran from crosses with the original stock also showed the angular type, modifiers must be involved. In some extracted bran groups the wings are still more angular at the tip and look like a transition to truncated wings. Again, modifiers seem to be involved, though we shall also meet with alleles which are characterized by the angular or dumpoid effect. The more rounded type resembles considerably the sex-linked mutant broad (br), though it is autosomal. A homozygous combination of both bran and br was made, which has an additive effect, still shorter and very broad, almost spherical wings. A very frequent feature of bran is upturned posterior scutellars. Further, some individuals occasionally have a small blister at the base of the wing. Their number may be increased by modifiers, as we shall see below, and, in addition, there is a combination of some bran and poi alleles which is characterized by such a blister (see below). Very rarely (1 in about 10,000) an individual looks like soft blistered (which contains  $svr^{po1}$ ), but these, when tested, prove to be nothing but pure bran. When bran first appeared, there was a tendency towards short bristles, which, together with the smaller size of bran males (not females), produced a Minute-like effect. Later the bristles became and remained normal.

Though bran is a recessive, sometimes the heterozygote has somewhat broader wings, a dominance effect found in many recessives (e.g., bs, ba, px). In connection with another problem these dominance relations were studied in detail. The results are of interest in view of the origin of bran both from px bl and from  $svr^{po1}$ . A large series of different derivatives of px bl and pointed were crossed with bran and the dominance condition for individual broods was recorded in four classes: no, little, more, and high dominance. None of the tester stocks contained bran, but one contained arc, which acts as an allele of bran, as will be discussed below. Table 47 contains the data. This table shows that all crosses with different  $svr^{po1}$  stocks show hardly any dominance of bran; all the different px bl derivatives exhibit considerable dominance, and in two cases all broods have rather high dominance (all crosses made simultaneously under identical conditions and with long inbred stocks derived originally from one pair). The cross containing arc exhibits the allelism, with the additional feature of a lower phenotype of only the females in 6 broods. Obviously, dominance modifiers are involved, which, however, have not been analyzed further.

The localization of the mutant gave the following results. Crossover was checked

for the two dominant loci Lobe (72.0) and Bristle (54.8). Further, a combination of bran with ll sp was obtained by crossing over, the strange phenotype of which will be described below. This combination permitted an exact localization, as table 48 shows. This puts bran at or near the arc locus (99.2), a localization which

TABLE 47

F<sub>1</sub>N × bran

N	Broods	Grades of dominance (number of broods)				Remarks
		0	1	2	3	
1. px bl II.....	12	..	..	12	..	
2. px bl III.....	10	..	10	..	..	
3. px bl r.....	10	..	10	..	..	
4. px from poi × poi.....	11	..	11	..	..	
5. px extr.....	10	..	..	10	..	
6. px bl sel.....	10	..	10	..	..	
7. px low.....	9	..	6	3	..	
8. a px sp blist from 4.....	10	..	..	..	10	Allele! In 6 broods only ♂, ♀ in class 1
9. svr <sup>poi h</sup> I.....	10	10	..	..	..	But a few class 1
10. svr <sup>poi h</sup> II.....	12	12	..	..	..	But a few class 1
11. svr <sup>poi h</sup> III.....	9	9	..	..	..	But a few class 1
12. svr <sup>poi</sup> I.....	12	12	..	..	..	But a few class 1
13. svr <sup>poi</sup> II.....	11	11	..	..	..	But a few class 1

agrees with the phenotypic data to be recorded, and with the salivary analysis, which shows a one-band deficiency in this region for one bran allele.

The mutant arc has broader, flapper-like wings which are either arched or up-turned. But the near-by locus plexus (100.5) also tends to have broadened, blunted wings. Bran was therefore combined in compounds with these loci and further tested opposite the Df(2)apx (or MI). It behaved like an allele of arc, but there are

TABLE 48

CROSSING-OVER TEST FOR bran

	No.	C.o.	Per cent	Locus
C.o. Bl-bran.....	376	134	35.6	90.4+
C.o. l <sub>2</sub> -bran.....	496	137	27.6	99.6
C.o. ll-bran.....	1338	109	8.14	98.56

a number of special features which will be discussed in another section below. On account of those features we did not speak of a<sup>bran</sup>, but of bran.

*Sex ratio.*—The sex ratio within bran is normal. One count of pair matings gave 710 females 690 males. Only one of the broods had a 2:1 ratio and therefore contained a sex-linked lethal. Crossing bran females with Oregon males, an average sex ratio of 1491 females 1384 males was obtained. This was based upon the distribution shown in table 49. Comparing these ratios with those studied in poi, we see that in the long inbred bran line the different rearrangements have largely disap-

peared, but that some of them are still left in the stock. It did not seem worth while to repeat the analysis.

*Combination with pointed.*—Another remarkable feature of bran is its already mentioned recombination effect with poi. In the majority of cases in which it first appeared it was in this recombination. The usual effect (see below) of bran and poi homozygous together is a wing called *soft blistered*. The wing is shorter and more or less pointed and contains in the majority of individuals, usually in all, a large blister. Frequently, the wing is carried at angles to the body. Viability is lowered,

TABLE 49  
SEX RATIOS bran  $\times$  Ore

	Sex ratio						
	Below 1	-1 3	-1 6	-1 9	-2.2	-2.5	-2 8
Broods.....	6	13	6	1	1	1	1

but the true breeding stock is easily kept except when px is present simultaneously, as will be shown below. A certain percentage of the soft blistered flies, varying from none to about 20 per cent, show either one or both wings truncated, in extreme cases looking like an extreme rudimentary. But neither dp nor r is involved. Many selections for these truncate types were made, but only once (see below) were they somewhat effective, and in this case it was not the ordinary bran which was present. (Different bran alleles behave differently in this respect; see below). Obviously, these truncated wings are the result of competition between the pointed and the

TABLE 50  
 $F_2$  (poi  $\times$  DIFF. bran)<sup>2</sup>

Class	+		poi		bran		soft blist	
	♀	♂	♀	♂	♀	♂	♀	♂
Number.....	258	223	141	150	40	50	67	51
Expected.....	♀ 379.5		♂ 355.5		63.3	59.3	63.3	59.3

broadening tendencies in development. This can be best demonstrated if bran is combined with lanceolate<sup>2</sup> (11<sup>2</sup>) by crossing over in the same chromosome. Such flies show at one end of a series of variations lanceolate, and at the other end truncate (dp or r-like) wings. The majority of individuals show all combinations of truncation with sharpening of the wing tips, different usually in right and left wings, in complete, more or less asymmetrical seriation from a lanceolate to a truncated wing. There is also a tendency toward arching in this combination. It ought to be added that both pointed and bran never show extravenation, though they are derived from a stock containing both px and bs. Soft blistered was synthesized by crossing both svr alleles to all the different bran stocks and breeding  $F_2$  for the homozygous recombination, expected in one-eighth of the offspring. The result of such an experiment is shown in table 50 (nos. 5554 ff). The ratio 3 not : 1 bran and 7 not : 1 soft blist are rather good, though bran seems to be a little less viable. This was observed



also whenever bran was segregated in other crosses. This combination of bran and poi is very useful for the discovery of such pointed alleles, which otherwise are hardly distinguishable, and also for the analysis of position effects at the *svr* locus, as will be shown below.

*The X chromosome.*—It ought to be added that in all synthetic or analytic crosses involving soft blistered the possibility for abnormal sex ratios exists when the pointed X chromosome introduces the different deficiencies, translocations, and transpositions studied above. They are actually obtained, as the following example shows. (This fact tends to show that the second chromosome of bran still contains the small translocation from 1→2 near the arc locus, which is actually visible in the salivary gland chromosomes.)  $Svr^{poi\ h}$  was crossed with bran, which simultaneously contained ll sp in the second chromosome.  $F_2$  showed the segregation presented in table 51.

TABLE 51  
( $svr^{poi\ h} \times \text{bran ll sp}$ )<sup>2</sup>

♀				♂			
+	poi	bran ll sp	soft bl sp	+	poi	bran ll sp	soft bl sp
499	272	81	95	445	117	37	41
Exp. 3	3	1	1	3	3	1	1

As before, part of the pointed individuals are practically normal. The female ratio for not bran : bran (bran = bran + soft bl) is 4.4 : 1, which is probably due to the differential viability of bran. But in the males the ratio is 7.2 : 1. If we take the female classes as normal, half of the poi, soft blist, and bran ll sp males are missing. The male X chromosome may be in part a result of crossover, with all the chances for lethal classes discussed above for pointed. The total sex ratio is, as so frequently with pointed crosses, near 4 : 3, namely, 1.37. In another backcross soft blist  $\times$  ( $N \times$  soft bl) the sex ratio was 189 : 92, one-half poi males in all classes missing. Another cross, (soft blist  $\times N$ )  $\times$  soft blist, had a sex ratio of 568 : 410 = 1.38, with males again missing in the poi classes.

Since the detailed analysis of these ratios has been presented for poi, only a single check will be reported, namely, for the transposition in the X chromosome of pointed in the cross ( $X \text{ ple} \times \text{soft blist}$ )  $\times X \text{ ple}$  (table 52). All classes with the left end of the poi X but without the section near miniature (*m*) (*m* is located between *v* and *wy*) of the same chromosome are lethal for males. Adding these and their reciprocal classes, we get 35 female 41 male crossovers with either only the right part of the poi X beyond white or the left end plus the *m* region of the same chromosome; but 34 females 0 males in the reciprocal classes, i.e., left end of poi without its miniature region. This one check may suffice.

*Modifiers.*—One type of modifier ought to be mentioned because it produces a phenotype which cannot be distinguished from a certain other one which genetically is altogether different, namely, a combination of bran with another poi allele. (The check is of course a cross with poi.) It was stated above that occasionally some bran individuals have a blister at the wing base. In this case the wing is sometimes just a little sinuated at the posterior margin, like a beginning truncation. This type

is based upon an autosomal modifier in the third or fourth chromosome derived from px bl, which seems to contain it regularly. In a series of  $F_2$  crosses (px bl  $\times$  bran)<sup>2</sup>, out of 1481 individuals 112 of both sexes had the type of wing described. This is roughly one-fourth of all bran segregants, indicating an autosomal modifier outside the second chromosome.

TABLE 52  
1673-77 ( $X^g/Cl B \times$  soft bl)  $\times X^g$

	$\Sigma$	
	♀	♂
+	82	71
$X^g$	17	..
y	..	1
w ec cv ct v m g f.	..	..
y w	2	1
ec cv ct v m g f.	..	..
y w ec	4	9
cv ct v m g f.	3	..
y w ec cv	8	7
ct v m g f.	7	..
y w ec cv ct	6	8
v m g f.	10	..
y w ec cv ct v	..	2
m g f.	6	..
y w ec cv ct v m	..	..
g f.	..	..
y w ec cv ct v m g	..	..
f.	7	3
y	..	1
y ec cv v	..	4
v	7	1
cv ct v m	1	..
y	..	1
y ec	..	1
y w ec cv ct	1	1
y w ec	2	..
g f.	3	..
ct v m	1	..
v m g	1	..
v m	1	..
$\Sigma$	170	112

*Origin.*—It was reported in the first section how the bran type first appeared simultaneously with pointed and rudimentary when the px bl line broke up (so-called mass mutation or upheaval). Later the same thing happened in a stock of a different px bl line. Since then bran has reappeared in various ways. Both px bl and poi stocks were checked all over again in large series of pair crosses, repeated from time to time, for the presence of bran (also poi in px bl), always with negative results. But rarely a single or a few soft blistered flies were found in  $svr^{poi}$  bottles, which, when checked, turned out to be homozygous for bran. Pure bran flies also

cropped up occasionally, i.e., flies in which bran had appeared as a mutant and pointed had simultaneously reverted to normal. The same thing has happened in constant and long inbred soft blistered stocks: a single bran fly was found by reversion of poi to plus; also in a stock with another poi allele. Further cases in which bran appeared in controlled matings will be reported and tabulated below, when we discuss the interrelation of all these happenings.

### C. FURTHER pointed AND bran ALLELES

In the course of the work, other alleles of pointed and bran appeared, some easily distinguishable, others only traceable by their combination effects with bran.

*The alleles svr<sup>poi bl</sup> and Bran.*—The allele svr<sup>poi bl</sup> if isolated can hardly be distinguished from svr<sup>poi</sup>; it is also a suppressor of speck. It is best recognized in the

TABLE 53  
Patt × (Patt × poi bl)  
[Patt =  $\underline{y}$ , bw, e, ey]

♀								♂							
Patt	bw e	bw ey	e ey	bw	e	ey	+	bw e ey	bw e	bw ey	e ey	bw	e	ey	+
19	12	7	9	32	36	3	39	10	13	18	18	19	17	21	23
plus + ey ♀ 42, ♂ 44 e + e ey ♀ 45, ♂ 35								bw + bw ey ♀ 39, ♂ 37 bw e + bw e ey ♀ 31, ♂ 23							

combination with bran, i.e., bran/bran, svr<sup>poi bl</sup> flies (both sexes) are not soft blistered with short wings, but have long pointed wings with a blister, or, sometimes, a singed place instead, and are occasionally even indistinguishable from poi. This allele (abbr.: poi bl, the combination with bran called pointed blistered or poi blist) is then more epistatic over bran than is svr<sup>poi</sup>.

Its origin is rather complicated. We mentioned, in the Introduction, the appearance in certain crosses of a type with soft spread plexate and blistered wings (it is actually pointed blistered + plexus). It will be analyzed later, together with the discussion of the original happenings. A few individuals of this type were obtained in the following cross: 5891 B svr<sup>poi</sup> × (svr<sup>poi h</sup> × svr<sup>poi</sup>), i.e., a backcross between the pure silver alleles, neither of which was known to contain bran, px, or svr<sup>poi bl</sup> when the cross was made. We shall later discuss some such crosses that produced a reversion to px, which had been absent before. This backcross produced 40 females 35 males pointed, 1 female 4 males soft blist px spread. A pair of the latter type was mated, with the result: 6063 B = 5891<sup>2</sup> soft, etc.; 41 females 48 males poi, 16 females 12 males like parents, 2 males plexus, 5 females 2 males pointed blistered. Pointed blistered turned out to be heterozygous px, was isolated without px, and has bred true ever since. It contained bran homozygous and the new pointed allele. Thus, px, bran, and the new pointed allele arose simultaneously in a compound of svr<sup>poi</sup>/svr<sup>poi h</sup>. The meaning of this will be discussed in a special section. The poi blist stock was derived from this case; but the new allele has been found to reappear repeatedly (see below).

Pointed blistered was bred for many years. It never showed the short and fre-

quently truncated wings of soft blist, but varied in the amount of blistering. The location of the blisters differs from that in the px bl stock. The blisters are situated, not above the connection of the posterior crossvein with the fifth longitudinal, but either behind the crossvein in the fourth posterior cell near the posterior wing edge, or in the fifth cell. Sometimes a singed-looking area replaces the blister. Genetic tests showed clearly that the bran contained in poi blist is the same as studied before.  $F_2$  from poi bl  $\times$  plus segregates typically into 6 plus and poi, 1 bran, 1 poi bl. If both poi blist and soft blist are introduced together by a compound female, some  $F_2$  segregates contain both soft bl and poi bl, thus showing the allelism of poi and poi bl. Otherwise the stock showed all the peculiarities of pointed with respect to modifiers and sex ratios, i.e., the different male lethal conditions described above.

TABLE 54a  
Patt  $\times$  (Patt  $\times$  bran)

♀								♂							
Patt	bw e	bw ey	e ey	bw	e	ey	+	bw e ey	bw e	bw ey	e ey	bw	e	ey	+
56	50	60	72	106	91	51	154	66	84	70	73	113	112	39	144
♀ plus + ey 205 e + e ey 163 bw + bw ey 166 bw e + bw e ey 106								♂ plus + ey 183 e + e ey 195 bw + bw ey 183 bw e + bw e ey 150							

One special feature was found for this allele which is worth mentioning. In making translocation tests with y, bw, e, ey (Patterson test) it was frequently noticed (see above, p. 319) that eyeless showed a lowered expressivity. We selected, therefore, a Patterson stock for high-grade eyeless, which gave more satisfactory results. But in crosses with poi blist we found the rather extreme results shown in table 53. The male segregation is normal (but for the usual tendency for smaller classes with increase of recessive mutants). But in the females the ey class is almost missing, and the bw ey and e ey classes are very small. But the size of the corresponding classes (ey-+, bw ey-bw, e ey-e) shows that the missing ey flies are contained in the corresponding class, e.g., in males 21 ey males + 23 plus males = 44, in females 3 ey + 39 plus = 42 females, etc. As the Y chromosome of the female is derived from the poi father, one might think of a  $Y \rightarrow 4$  translocation with an effect comparable to Dubinin's cubitus interruptus effect. But a similar test with bran (not derived from poi bl) gave the results found in table 54a. Again while taking into account the different viability of multiple recessives, we find in the females little, if any, suppression of ey except in the plus and ey class. But now we find the same also in the males, which in this case do not have the poi X chromosome and have the Y derived from the Patterson stock. Thus it seems that the poi bl X prevents the ey suppressor action. Tests with cubitus interruptus were negative; hence we are inclined to assume that a suppressor action by some modifier and not translocation is involved in both cases.

A few years later the test for poi bl was repeated and only ey was checked, i.e., y, ey  $\times$  (y, ey  $\times$  poi bl). The classes not ey and ey were:

not ey: 196 females 144 males; ey: 22 females 57 males

The suppressor action which now worked in both sexes, though more so in females, then, is typical for the poi bl stock. This suggested a repetition for bran and for standard pointed. Soft blistered was used, which contains both. Simultaneously, another bran stock (marked 5087), which had arisen later, was tested. The results are contained in table 54b. As before, some of the ey effect was visible, but nothing comparable to the action of the poi blist stock. Therefore poi blist was tested over again, with the same results as before, which thus are proved typical. The explanation has been presented above, i.e., a dominant eyeless suppressor action located in the second and third chromosomes of this stock. This action seems to be additive when both second and third chromosomes from the poi bl stock are present in heterozygous condition. Furthermore, whereas the action may be found in different cases either in females only, or more extremely in females or in both sexes, it must

TABLE 54b

Cross	♀							
	Patt	bw e	bw ey	e ey	bw	e	ey	+
Patt × (Patt × soft bl).....	19	34	18	20	30	19	15	49
Patt × (Patt × 5087 bran).....	65	63	75	63	85	59	45	97

Cross	♂							
	bw e ey	bw e	bw ey	e ey	bw	e	ey	+
Patt × (Patt × soft bl).....	16	10	15	13	17	16	20	42
Patt × (Patt × 5087 bran).....	51	72	66	53	72	56	50	65

be assumed either that the X chromosome from poi, etc., more or less counteracts the suppressor effect, or that this effect tends—in a variable way—to work better in the female developmental system. If a suppression of eyeless by dominant suppressors is involved, the large not ey classes must contain many ey/ey individuals with suppression. Therefore a large number of RF<sub>2</sub> females from these classes were mated to brothers with eyeless (and bw or e). This was done for all cases in which the phenomenon was observed. The clearest results were expected in the case of poi blist, where the most extreme suppression of ey was found. F<sub>3</sub> should in this case contain a mixture of broods from ey/ey and ey/+ mothers. If we assume for F<sub>3</sub> about the same effect as found in F<sub>2</sub>, we can calculate that the ey/ey mothers will produce about equal numbers of not ey and ey daughters; the ey/+ mothers about 3 not ey : 1 ey daughters. Among 25 broods, 13 had high ratios, altogether 337 not ey : 133 ey = 2.6 : 1; 12 broods had ratios near normal or even with an excess of eyeless, namely, 222 not ey : 275 ey = 0.8 : 1. We consider this a proof of the presence of suppressors in the second and third chromosomes. The other question, whether the absence or smallness of the effect in males is due to the specific X chromosome, is answered by a cross reported above (p. 318) (poi × bw, e, ey) × bw, e, ey. Half of the sons do not have the poi first chromosome, but they show the ey suppressor effect as much as the females do. (In this case, however, crossing over is possible). Therefore we must conclude that the poi X is actually responsible for the absence or covering of the effect in males. In order to make a comparison easier, we tabulate in table 55

all the backcrosses of the type under discussion and arrange the classes so that not-ey and ey classes follow each other in four pairs. In this table also, crosses are included which will be discussed below in connection with the additional ey domi-

TABLE 55  
BACKCROSSES INVOLVING ey SUPPRESSION AND DOMINANCE

Cross	Females							
	bw e	bw e ey	bw	bw ey	e	e ey	+	ey
Control Oregon.....	73	68	87	58	81	62	89	73
Patt × (Patt × poi).....	57	48	62	41	59	43	63	47
Patt × (Patt × poi h).....	15	14	24	12	20	17	36	14
Patt × (Patt × pois).....	102	91	172	57	123	95	165	76
Patt × (Patt × poi blist).....	12	19	32	7	36	9	39	3
Patt × (Patt × bran).....	50	56	106	60	91	72	154	51
Patt × (Patt × bran 5087).....	63	65	85	75	59	63	97	45
Patt × (Patt × soft blist).....	34	19	30	18	19	20	49	15
Patt × (Patt × poi:dp).....	22	55	63	24	42	36	67	35
Patt × (Patt × sl:dp).....	2	19	23	13	13	8	29	11
Patt × (Patt × px bl II).....	68	105	127	36	89	36	132	69
Patt × (Patt × px bl 4856).....	51	52	60	48	38	47	83	36
(poi × bw e ey) × bw e ey.....	157	128	221	107	185	86	227	99
(poi × bw e ey) × bw e ey.....	..	..	..	..	..	..	..	..
only ♂♂ not poi								
bw e ey × (px bl × bw e ey).....	53	66	88	58	83	79	116	59

Cross	Males							
	bw e	bw e ey	bw	bw ey	e	e ey	+	ey
Control Oregon.....	123	94	85	123	114	80	123	80
Patt × (Patt × poi).....	34	44	55	56	58	40	76	66
Patt × (Patt × poi h).....	16	14	23	17	18	21	22	30
Patt × (Patt × pois).....	44	90	129	117	79	77	191	111
Patt × (Patt × poi blist).....	13	10	19	18	17	18	23	21
Patt × (Patt × bran).....	84	66	113	70	112	73	144	39
Patt × (Patt × bran 5087).....	72	51	72	66	56	53	66	50
Patt × (Patt × soft blist).....	10	16	17	15	16	13	42	20
Patt × (Patt × poi:dp).....	2	69	39	25	36	43	80	45
Patt × (Patt × sl:dp).....	10	104	63	71	43	60	137	50
Patt × (Patt × px bl II).....	80	67	102	89	111	85	135	80
Patt × (Patt × px bl 4856).....	62	53	66	48	65	43	84	44
(poi × bw e ey) × bw e ey.....	63	130	156	118	125	67	142	88
(poi × bw e ey) × bw e ey.....	54	101	103	58	108	46	97	46
only ♂♂ not poi								
bw e ey × (px bl × bw e ey).....	40	57	105	43	58	54	132	37

nance effect already mentioned, but most extremely represented in a stock to be analyzed later. Many different degrees of the effect (including absence of the suppressor in the second chromosome) are represented.  $F_3$  tests for cases with a less extreme suppressor action were made in the same way as described above for poi blist. In a set of 53  $F_3$  broods in which the expectation for the female offspring of suppressed ey/ey and ey/+ was about 0.8 not eyeless to 1 eyeless and 2 not eyeless

to 1 eyeless, respectively, two clear-cut groups were found, namely, 32 broods with 559 not ey 235 ey = 2.4 : 1 and 21 broods with 357 not ey : 390 ey = 0.9 : 1.

We have already stated that the bran in poi bl which had originated simultaneously with this poi allele, just as the original poi had originated together with bran, behaved like ordinary bran when extracted or recombined with *svr<sup>pot</sup>*. But twice this bran exhibited unusual behavior. In the first case, an  $F_2$  (poi bl  $\times$  ll sp)<sup>2</sup> showed

TABLE 56  
3125 (poi<sup>bl</sup>  $\times$  ll sp)<sup>2</sup>

	♀	♂
+ and poi . . . . .	63	94
bran . . . . .	9	13
ll sp . . . . .	19	11
ll (sp) poi . . . . .	26	5
poi bl . . . . .	23	16

an abnormal segregation in so far as the bran class was too small, namely, no. 3725 (poi bl  $\times$  ll sp).<sup>2</sup> Expected: 4 + and poi, 1 each ll sp, bran, ll (sp) poi, bran poi = poi bl (results in table 56). The almost complete absence of ll (sp) poi males has been explained before by the translocation 1 $\rightarrow$ 2 near bran, which is derived from the ancestors of poi bl. But as bran is at least as viable as poi bl (though reduced in viability), the small number appeared significant.  $F_3$  was therefore bred from a bran pair. The result is detailed in table 57. It shows—apart from the sex ratio—that the mother was heterozygous for poi, and further, that one of the parents had been heterozygous for bran though phenotypically bran. Also, most bran individ-

TABLE 57  
4517. bran  $\times$  bran FROM 3725

	♀	♂	
+ . . . . .	63	17	Bristles minute
bran . . . . .	50	17	
poi . . . . .	..	18	
soft bl . . . . .	..	20	

uals showed a "Minute" type of short bristles. Further, no poi bl males, but soft blistered males, segregated. Obviously, a heterozygous condition of bran had arisen, with complete dominance and a bristle effect in the homozygote (compound) with bran. This compound together with poi produced soft blistered instead of poi bl wings. There is a strong suspicion that a bran deficiency had arisen of a size giving a kind of Minute effect including dominance, but viable opposite bran. A subsequent generation bred from these bran flies, which then are supposed to be compounds of the new and old bran, segregated into  $\frac{1}{2}$  bran and  $\frac{1}{2}$  almost bran, the latter with M bristles (4701). Another mass generation from this (4965) segregated again ll sp, showing that the incomplete bran in 4701 were heterozygous Bran/ll sp, and therefore that the parents bran 3725 had also contained Bran/ll sp and Bran/bran.

The final analysis of this new dominant Bran was prevented by a strange co-

incidence. In the last generation, 4965, a single crossover female *ll sp* and *dumpy* wings, also *dwarfish*, was obtained, but it was already fertilized when found. From this a stock was obtained, in 4 generations, homozygous for *ll sp* and *bran* in the

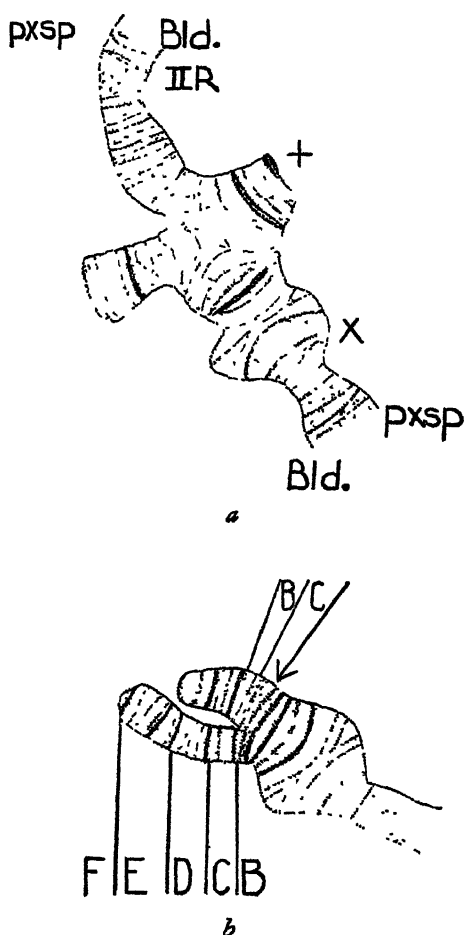


Fig. 1. Salivary gland chromosomes of  $T(1,2)Bld$  heterozygous with *px sp*. *a*. The tip of 1 and the free end of 2R unite into the typical cross-shaped figure. *Bld* = X from *Bld* with attached tip at 2 of left. Upper *px sp* = second chromosome from *px sp* synapsed at left with translocated part from *Bld*-X. *Bld*IIR = second chromosome of *Bld* with attached tip of 1, synapsed at right with X from *px sp*. *b*. The tip of X in *Bld*(1)Df. Above, the tip of normal X(1,ABC); below, the attached end of 2(80, B-F). M. Kodani del.

same chromosome, the phenotype of which was described above. Assuming that this was the new dominant *Bran*, the stock was kept for further tests. When the tests were made, it turned out that the stock contained the ordinary recessive *bran* and that the dominant had been lost. We shall later return to these crosses, since simultaneously with the dominant *Bran* there appeared also other mutants.



Once more, what probably was the same dominant Bran was produced after out-crossing *poi bl*, namely, to Blond translocation (Bld) in connection with the localization of bran. The overlapping of phenotypes requires a more detailed report, together with a few data on Bld which are missing in the literature on this translocation. Blond translocation is a reciprocal translocation between the right end of the second chromosome and the tip of the first. The breaks as studied by Bridges are reported in *DIS* 9, and text figure 1 illustrates the cytological facts. The tip of the first chromosome includes the *svr* locus. In the second chromosome the translocated piece does not include the bran locus, but the break is so near it that a cross-over combination could not be obtained. The balanced Bld is known to segregate females and males with Bld(2) deficiency which have normal X chromosomes and a second chromosome with the translocated tip of X; Bld(2)Df is therefore simul-

TABLE 53  
CROSS: Oregon  $\times$  Blond

No.	♀ Bld and ♂ +	(2)Df ♂	(1)Df ♀
4592.....	126	10	2
4593.....	108	12	6
4594.....	85	18	21
4595.....	84	5	5
4596.....	81	23	..
Σ.....	484	68	34
Exp.....	484	242	242

taneously duplicated for the tip of X. Bld(2)Df are small individuals with spread and plexated wings, short bristles, and rough eyes. Bld(1)Df with two normal second chromosomes and one X with the translocation from II is therefore deficient for X but duplicated for the end of II; only females survive, males being lethal. The Bld(1)Df females are very large, blond, with very short bristles (varying from half length practically to absence, and have extremely broad wings, caused mainly by the enlargement of the fifth cell; sometimes the wings are arched; notching at the tip is sometimes found, also inflation of the wings. There is, further, a tendency toward abnormal tarsi and large rough eyes. These females hatch about 3 days later than their sisters. Bld(2)Df females are very rarely fertile, the males more frequently, and a considerable number of combinations with these males could be made. Bld(1)Df females are usually sterile, but offspring was occasionally obtained, the number varying from 1 to 20-odd, with many dead pupae in the bottle. Furthermore, the viability of both deficient types is poor, and they rarely appear in the expected numbers and frequently are completely absent. They are especially sensitive to the composition of the food. A special test made under good conditions and with special checks upon late hatchers produced the broods shown in table 58.

A number of reciprocal crosses between Bld and *poi bl* gave the expected results. But one cross *poi bl*  $\times$  Bld no. 3922 gave a very unexpected offspring; all daughters looked like Bld(1)Df, not so large as usual and less abnormal-looking, but otherwise exhibiting the broad wings and short blond bristles. All sons were  $\pm$  pointed ( $\pm$

meaning varying expressivity). In the next generation, females and males of the Bld(1)Df type appeared, though real Bld(1)Df males cannot survive. The males were also blond, broad-winged, and short-bristled, but rather small. A stock was established which bred true for females, the males segregating into the new blond type and soft blistered, when bred from blond pairs. This showed that poi and bran from poi blist were present in the latter males. Much work was done to find out what had happened. It turned out that neither the females nor the males were Bld(1)Df, which they resembled so much, but simply Blond translocation (balanced) but heterozygous for a bran allele. In this case again, Bran was dominant and made the Bld flies look like the (1)Df. In addition, the Minute-like tendency to short bristles which we met before with dominant Bran was present in this case, so that the Bld(1) deficiency effect was almost completely imitated, except for size. Unfortunately, a helper lost the stock before the salivaries could be tested, but there can be no doubt that Bran had appeared, probably as a larger deficiency. I repeat again that many similar crosses had only the expected results with recessive bran, which did not cross over into the Bld second chromosome and thus could not become homozygous. We shall return to these crosses, which also produced other mutants. Possibly the same dominant Bran appeared a third time in one of the mass changes in a px bl bottle. It will be discussed in a later section, in which an analysis of these happenings is to be attempted.

*The alleles svr<sup>poi</sup> and bran<sup>2</sup>.*—The new allele of svr<sup>poi</sup> was derived from the allele poi bl and, characteristically enough, again from a cross with Blond translocation, a sister cross of the one just reported. The expectation for F<sub>2</sub> (poi bl × Bld)<sup>2</sup> bred from Bld F<sub>1</sub> ♀ and poi F<sub>1</sub> ♂, both heterozygous for bran (from poi bl), is 1/4 ♀♂ Bld, 1/4 ♀ Bld(1)Df with homozygous bran (♂ lethal), 1/4 ♀♂ Bld(2)Df with poi, 1/4 ♀♂ poi blist. The actual result was: 10 ♀ 20 ♂ Bld, 1 ♂ Bld (2)Df, 3 ♂ of a new type: broad, very angular wings with blisters. The absence of most of the (1)Df and (2)Df is a frequent occurrence. But the absence of 1/4 of poi blist ♀ and ♂, supposed to be at least as viable as the Blond translocation, is surprising, and has obviously to do with the appearance of the few males of the new type. These males were crossed to y and all sons were pointed; in F<sub>2</sub>, 1/4 of bran flies segregated in both sexes, and most of the bran males were blistered like the grandfather. The blister in this case has a tendency to be located near the base of the wing. The type has since been bred as a stock without change (marked 5121 broad blistered). It will be shown below that a new bran allele also is involved, bran<sup>2</sup>. In both sexes the wings look like exaggerated bran, i.e., the tip is very broad and angular (therefore poi sq = square), with a tendency toward a beginning truncate. The majority of the individuals are blistered as described, but some are not. A similar blistering was described above as an occasional modification of bran. The new combination differs, however, from ordinary bran in the blunter wings and tendency to truncation. The new poi<sup>2</sup> thus is hypostatic to bran and shows its presence only in the blistering effect.

Checks showed that the Blond translocation had nothing to do with the new type. The homozygous bran which the new stock contained seemed to be the same (see below) as standard bran as tested in F<sub>1</sub> with +, bran, and arc. But it turned out later to be a new allele, bran<sup>2</sup>, which shows a one-band deficiency in the salivaries. The bran<sup>2</sup> svr<sup>poi</sup> stock (abbr.: bran blist) contained also some modifier for the

bran expression. The compound  $Df(2) a/bran^2$  (from bran blist) has hardly any exaggeration effect, but in the backcross  $Df \times (Df \times bran \text{ blist})$  two types of compounds  $bran^2 Df$  appear in equal numbers, one like the  $F_1$  compound, one with very broad, frequently nicked wings and much shorter bristles. As only one combination of the second chromosome is possible, the difference is based upon another modifier, which must be autosomal, since all males have the same X chromosome derived from the tester stock. A backcross  $bran \text{ blist} \times (Df \times bran \text{ blist})$  shows the same modifying action. Among the Minute females ( $Df/bran^2$ ), two types, a lower and an exaggerated one, are found. The same applies to the males, all of which have the same X chromosome. Among those with the  $Df/bran^2$  compound (Minutes) also, a normal and a higher type are found, the latter with truncated blistered wings.

Thus the peculiar type of bran blist must be mostly based upon the pointed chromosome, which gives another combination effect with bran than the other alleles studied thus far. A first check is to introduce standard  $svr^{poi}$  into a combination with  $bran^2$  from bran blist. This ought to give the typical soft blistered; which it does:

Control =  $(bran \times bran \text{ blist})^2$  = all ♀♀ bran,  $\frac{1}{2}$  ♂♂ ditto,  $\frac{1}{2}$  bran blist  
 Test =  $(poi \times bran \text{ blist})^2$  :  $\frac{9}{16}$  ♀♀ poi,  $\frac{1}{8}$  ♂ bran blist,  $\frac{1}{8}$  ♂ soft blist  
 Obtained: 72 ♀ 68 ♂ poi  
               7 ♀ 9 ♂ bran blist  
               20 ♀ 10 ♂ soft blist

If another locus in the X chromosome were involved, both crosses would have the same chance for crossing over, and thus some soft blist ought to appear in the control—which was not the case. Thus we have to assume a new allele of poi, namely,  $svr^{poi''}$ , which, alone, has the same phenotype as pointed, but gives a different combination effect with homozygous  $bran^2$ . (Its sp suppressing effect is the same as in  $svr^{poi}$ ). For salivaries see below. As has been repeatedly stated, it was later found that the bran contained in this bran blist stock was not the standard bran, but a new allele  $bran^2$  which had arisen simultaneously with the poi allele. The proof will be furnished in the following section, where we shall meet with other new bran alleles.

The alleles  $svr^{poi''}$  = soft and  $bran^1$ ,  $bran^2$ ,  $bran^{ab}$ .—The recognition and analysis of the allele  $svr^{poi''}$  has presented the greatest difficulties and involved a huge amount of work. The reason is its variable expressivity and its combination effects with a new allele of bran which originated simultaneously. This silver allele arose at one time within the plexus blistered stock without any visible effect, but became visible after outcrossing. In the course of the many years that this work proceeded, the px bl stock was tested over and over again in a large series of pair crosses with pointed and bran for the presence of these mutants, always with negative results (see below for details). The last extensive checks were made in 1942. But in a series of crosses  $px \text{ bl} \times N$  (N meaning different test stocks) made during the first months of 1938, the  $F_1$  males with the X chromosome from px bl frequently had soft-textured, pointed-looking but not actually pointed wings, a type which could be isolated and bred as a sex-linked recessive. They were also pale and had the sp suppressor action of the poi alleles. At the same time, another type, called slender, with wings like lanceolate (II, second chromosome), also sex-linked, as it seemed, segregated from similar crosses and could be isolated. It, too, had a tendency to soft-textured wings. Both types were isolated later again from other crosses. Their analysis was made possible mostly through the compound effects with bran. It turned out that "slender"

has the same pointed allele as soft wings,  $svr^{poi}$ , plus a new and weak allele of bran in the second chromosome, which has very little effect in the combination, i.e., is almost hypostatic.

This  $svr$  allele is of special importance because it was found at one time to be widespread in one of the unselected  $px\ bl$  stocks (called  $px\ bl\ II$ ), thus showing that a pointed allele could originate here without becoming visible. (It disappeared later.) Furthermore, the simultaneous origin of a bran allele in  $px\ bl$ , again too weak to become visible by itself, is of importance. The facts must therefore be reported in more detail.

Soft wings are recognizable sometimes only by a certain sheen. In most cases the inner edge of the wings tends to be crumpled, and the wing tip is more or less

TABLE 59  
PRESENCE OF THE ALLELE  $svr^{poi}$  IN  $px\ bl\ II$ , MARCH, 1938  
 $F_1\ px\ bl \times \text{SOMETHING}$

Mother	Number of $F_1$ broods with			Other ratios $\sigma^+$ + : soft	All soft are folded	Most soft are folded
	$\sigma^+$ normal	$\sigma^+ \frac{1}{2}$ soft $\frac{1}{2} +$	all $\sigma^+$ soft			
$px\ bl$ .....	4	4	10	38 : 9 36 : 14	1	1
$px\ bl\ blist$ .....	5	5	22	47 : 15 32 : 11 48 : 18	..	2
Total.....	9	9	32	201 : 67		

pointed, which might easily be mistaken for the mutant pointed. (Both are pale, like all  $svr$  alleles.) In more extreme cases one wing is more or less folded. In the most extreme case both wings are rolled up like a wet towel. Frequently, a few individuals of this latter type are found among others; but in other instances every male without exception is of this extreme type. It is possible that these different types owe their origin to external conditions, though the frequent occurrence of a definite type seems to suggest a genetic causation by modifiers. Flies with soft wings tend to stick to the walls of the bottle, a fact which has to be reckoned with if counts are made. The soft individuals tended originally to have longer, softer bristles, a character which later disappeared. But in crosses with this allele, bristle abnormalities, such as V-shaped or forked bristles, are rather common.

The distribution of this allele in the  $px\ bl\ II$  stock in March, 1938, is recorded in table 59 for  $F_1$  males of pair crosses  $px\ bl \times \text{something}$ .

As the presence of the "soft" character is not visible in  $px\ bl$  (the pale color not being completely reliable in  $px\ bl$  females, better in males), the  $px\ bl$  males may be tested by crossing to attached X females. A series of 10 males from the same population thus tested gave normal males only once; in all other cases all were soft. Among these 9 broods, only 1 had ordinary soft wings; in 5 all wings were folded; and in 3 the majority were folded. All were pale. Heterozygous females are always normal, showing the character to be recessive. Obviously, the homozygous plexus

prevents the soft wing character from becoming visible. This might mean only that the strong plexation acting as a reinforcement of the wing makes the soft texture invisible (but see below), without affecting the pale color.

These results show that the majority of females in the population and almost all males were at that time homozygous for this recessive character; further, that homozygous normal females were very rare. (Cr.  $\frac{1}{3}$  of all cases.) In the px bl females the condition which produces the extreme folded type is relatively rare, whereas it is present in the majority of the males. Out of 14 heterozygous px bl females, 9 produced a 1:1 ratio of normal and soft-winged males, but 5 an exact 3:1 ratio. As only in two of these cases were about one-half of the males missing, this ratio is obviously due to a recombination with a "modifier." Actually, some of the not soft (though pale) males of this group transmitted soft in  $F_2$ , thus showing that only the phenotype was modified. This is, then, the same situation as was presented above for the pointed-wing phenotype of *svr<sup>poi</sup>*. The table shows further that blistered and not blistered mothers produce the same types of sons.

$F_2$  from soft males gave, according to expectation,  $\frac{1}{2}$  plus,  $\frac{1}{2}$  soft of both sexes, and the recombination with homozygous px did not influence this condition. This is remarkable, as in the px bl stock the plexation obviously prevents the visibility of soft. But we shall see later that in  $F_2$  from px bl crosses the low-type ordinary plexus appears, and not the strong plexation of px bl. This may mean that low plexation does not prevent visibility of soft, but it may also mean that the changed genetic situation after outcrossing (see below) is responsible for the visibility of soft with plexus.  $F_3$  and  $F_4$  from soft parents with or without px bred true.

The details of the data summarized in table 59 show that there is an additional relation between soft and the sex ratio, which, as will be shown below, is frequently abnormal in  $F_1$  with px bl. In the cases in which px bl was homozygous for the absence of soft in the X chromosome, the  $F_1$  sex ratio was normal, i.e., 378 ♀:352 ♂ = ca. 1:1. When the px bl mothers were heterozygous for soft, a ratio of 773 ♀:561 ♂ was found, which is almost exactly 4:3, suggesting that 1 out of 4 males does not survive. The average ratio of normal to soft males in this case was 2:1 (298:149, not counting unreliable cases). The  $\frac{1}{4}$  of missing males thus belongs to the soft class. In the cases in which all  $F_1$  males were soft and therefore the mothers were supposed to be homozygous for soft, the sex ratio in bottles carefully checked for males sticking to the bottle was 2150:759 = 2.8:1.

These sex ratios clearly indicate the presence—later proved—of the same translocations producing lethal classes as in pointed, which, then, seem to be essential also in the production of the new allele *svr<sup>poi</sup>* (see below). Also, the same condition of the male X as in pointed (explained by a transposition) was found. The cross  $y, bw, e, ey \times px\ bl$  gives soft or normal  $F_1$  males and a normal sex ratio. The normal sex ratio for soft males indicates that px bl males containing a soft X chromosome are free from a lethal effect, seen when the X was derived from a female. But it cannot be excluded that the normal sex ratio is produced by the presence of both female and male lethal classes, the former caused by the presence of two foreign Xs.  $F_1$  males of this cross, normal as well as soft and folded, were backcrossed to  $y, bw, e, ey$ , and again the sex ratio was perfectly normal in all cases, as well for the sum total as for the segregating classes. This shows the same condition in soft males as was encountered before in pointed. The soft stock was kept for many years, but its

phenotype changed toward something like pointed. But in outcrosses all the typical features remained, as will be reported. We repeat that all soft individuals are also pale and that they have also the speck suppressor action.

The type called slender, i.e.,  $svr^{poi} +$  a new bran allele, was obtained in a different way, but also always out of crosses with px bl II performed in the same period, though not always involving the px bl X chromosome. Slender has a phenotype which is easily recognizable, especially when it is cleansed from modifiers by outcrossing. The wings resemble those of lanceolate<sup>2</sup>, though they are not as narrow. They are very pointed at the tip, and the posterior edge shows the inward curving at the end of the fifth vein which characterizes lanceolate. The wing appears longer than usual because of its narrowness.

Slender appeared first from crosses involving px bl but made so that the X chromosome from px bl was not present, namely:

1st gen. no. 668: + (Florida) × px bl ♂

2d gen. no. 913: bs × F<sub>1</sub> ♂, i.e., a male with an X chromosome from Florida, otherwise heterozygous with px bl. All offspring were giant with a normal sex ratio (for giant see later)

3d gen. no. 1147 = 913<sup>2</sup> giant: ♀♂ giants and intermediates, ♂ also dwarf; sex ratio normal

4th gen. no. 1264 = 1147<sup>2</sup> intermed.: 25 ♀ giant 9 ♂ intermed. (one dwarf) and soft wings

5th gen. no. 1363 = 1264<sup>2</sup> giant × soft: many ♀ normal, 2 ♀ 3 ♂ ski wings

6th gen. no. 1410 = 1363<sup>2</sup> ski: only 30 indiv., half ♀♀ are slender, all ♂♂ soft

7th gen. no. 1436 = 1410<sup>2</sup> slender × soft: all slender; breeds true (stock old slender)

A second appearance of the allele had similar features:

1st gen. no. 1935: px bl II × + (Oregon) part males soft; sex ratio 4 : 3 (made 1938 when soft was contained in px bl II)

2d gen. no. 2145 = 1935<sup>2</sup>: normal segregation, many ♀♂ soft wings

3d gen. no. 2145<sup>2</sup>: many giants

4th gen. no. 2297 = 2145<sup>2</sup> giants: size variable, half ♀♂ slender

5th gen. no. 2390/91 = 2297<sup>2</sup>: slender breeds true (stock 2297 slender)

In both cases, slender was derived from px bl II at a time when it was known to contain the soft allele. In both cases, the production of giants preceded the appearance of slender, i.e., a bran allele + soft. In the second case, soft was already present in the X chromosome derived from px bl. In the fourth generation via giants, slender appeared. Giant, then, had to do with the appearance of a new bran allele present in slender. In the first case, however, the X chromosomes were derived from bs or Florida stock. Nevertheless, in the fourth generation the X chromosome allele soft appeared from giant parents, and two generations later slender came from somewhat unusual parents (ski wings), showing that the bran allele had appeared again. The soft mutant, then, must have been produced here via an autosomal condition derived from px bl, a condition involved in the production of the giants as well as of the new bran allele (crossing over in the X chromosome being also excluded).

The new bran allele, bran<sup>1</sup>, was found to be present in slender when slender and soft were compared in outcrosses with arc, bran, and the different poi alleles combined with bran. It turned out that soft alone behaved in crosses with arc like pointed, i.e., arc was simply recessive. But slender × arc produced flies with broader wings which could be described as almost bran, and further generations showed the same compound effect, though the homozygous new allele without soft could hardly be distinguished from normal. The same compound effect—almost bran—was obtained in crosses slender × bran (but not soft × bran). We saw, when studying the

TABLE 60  
CROSSES WITH soft (svr<sup>poi</sup>) and slender (bran<sup>1</sup>/bran<sup>1</sup>, svr<sup>poi</sup>)

No.	Cross	♀							♂							Remarks				
		+	soft	poi sing	poi	al	bran	soft blist	dp blist	poi blist	+	soft	poi sing	poi	al		bran	soft blist	dp blist	poi blist
6025	al × poi	..	..	..	All	..	..	..	..	..	..	All	..	..	..	..	..	..	..	♀ + are almost bran
6026	al × bran	81	..	..	..	..	..	..	..	..	..	31	11	..	..	..	..	..	..	♀ + are almost bran
6027	al 2300 × bran	33	..	..	..	..	..	..	..	..	..	9	11	..	..	..	..	..	..	♀ vary between soft and slender
6028	al × soft	..	10	21	..	37	..	..	..	..	..	62	1	..	..	..	..	..	..	♂ ± poi
5512 ff	soft × bran	324	..	..	..	..	..	..	..	..	..	179	10	..	..	..	..	..	..	8 ♀ al have one wing al blist; 3 ♀ al other wing poi sing
6051	soft × bran blist = bran <sup>2</sup> /bran <sup>2</sup> , poi <sup>2</sup>	..	..	..	..	30	..	..	1	..	..	19	4	..	..	..	..	..	5	
6093	(al × poi) <sup>2</sup>	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	From poi via soft to slender, not classifiable
6095	(al × bran) <sup>2</sup>	24	15	..	22	..	13	10	..	..	34	17	..	..	6	10	12	..	..	al are very slender and singed; + are almost bran
6096	(al × bran) <sup>2</sup> ♂ sing.	41	19	..	..	19	10	12	..	..	36	13	..	..	11	4	11	..	..	al are very slender and singed; + are almost bran
6098 ff	(al × soft) <sup>2</sup>	..	..	76	..	88	..	..	..	..	..	..	19	..	82	..	..	..	..	♀ all al and al sing; ♂ more like poi
5701/02	(soft × bran) <sup>2</sup>	81	25	17	..	..	17	19	..	..	84	49	8	..	..	14	20	..	..	♀ poi sing = al sing; ♀ poi bl = al blist;
7094 ff	(soft × bran blist) <sup>2</sup>	..	..	22	..	95	..	39	..	37	..	..	15	..	97	..	20	..	9	♀ soft bl = bran bl + soft bl; al contains also soft
7097	(soft × bran bl) <sup>2</sup> ♀ ♀ dp	..	..	3	..	30	..	5	8	10	..	..	4	..	28	..	10	2	6	poi contains ± poi
5715/16	(soft × bran bl) <sup>2</sup>	..	..	9	174	43	..	23	..	..	..	..	5	134	49	..	30	8	..	

combinations of  $svr^{poi}$  and  $svr^{poi}{}^{aa}$  with homozygous bran, an inclination toward the formation of truncated wings. Actually, the slender type is nothing but a beginning of truncation in soft pointed wings; sometimes one wing of slender flies is intermediate to truncated or actually like dumpy (see below). Slender, then, is homozygous bran<sup>1</sup> with the soft allele of  $svr^{poi}$ , bran<sup>1</sup> homozygous being practically normal. (Consult the table of phenotypes, pp. 388-389.)

The crosses between soft and slender with bran show another phenomenon which must be based upon the soft allele, as it is identical in both crosses. A part of the bran/+, poi s/poi s or bran<sup>1</sup>/+, poi s/poi s, or bran/bran<sup>1</sup>, poi s/poi s females and also hemizygous males have a new wing type which we marked as "very slender singed." The wing is still narrower and more indented than is slender, and has near the tip a singed or crumpled-looking spot. This is not a dried-out blister, as the fresh wing shows, but something which might be described as a rudimentary blister. This means that the soft allele acts upon a heterozygous bran, producing almost a blister (the other alleles require a homozygous bran). This, then, is another distinguishing feature of the soft allele. The numbers of singed flies of the constitution bran/+, poi s are rather variable. Usually about one-third of the females and only a few males show the type. Sometimes almost all females and up to one-half males exhibit the type. Probably modifiers are involved. The same singed effect appears also in the heterozygote with bran<sup>1</sup>, i.e., when soft and slender are crossed, producing bran<sup>1</sup>/+, poi s flies. We shall meet below with a similar phenotype based upon a different allele.

The cases reported above were not the only ones in which the mutant bran<sup>1</sup> appeared, visible as slender in the combination with soft (see below). Thus, once after outcrossing soft with bran, F<sub>2</sub> contained, in addition to the expected classes, pointed singed flies (no. 5704) which, inbred, segregated slender but never bran in tests over many generations. The singed flies, therefore, could not have been bran/+, i.e., with the bran crossed into the parents. Tests with bran showed that actually bran<sup>1</sup> was present, which has no clear homozygous effect without soft. Either bran<sup>1</sup> had appeared as a mutant, or bran had mutated into bran<sup>1</sup>.

If soft is combined with homozygous ordinary bran, again the old soft blistered type is found. But it tends to contain a much larger number of truncated blistered flies, up to a short rudimentary wing, than is found in soft blistered involving the other poi alleles + bran. Only a few examples of these and similar crosses are given in table 60. All types reported formerly and in table 60 were further tested in following generations and different backcrosses and found to be of the constitution just assigned to them. A few additional features are visible in the table. As a rule, males tend to show all characters in a lower degree than the females. When females are soft, males may be somewhat like pointed, or even more normal. When singed appears, the males show it in smaller numbers. (Of course, singed and not singed flies of the same class were tested with respect to having the same genetic constitution.)

The table contains also crosses between soft and bran blistered. The latter was homozygous bran<sup>2</sup> together with the allele  $svr^{poi}{}^{aa}$ , a combination which shows broad and square wings with a basal blister. All former tests had apparently shown that the bran in bran blist is the normal bran. But the crosses with soft reveal that another allele of bran is involved (as already anticipated above). As early as F<sub>1</sub> some



females and males have wings like slender, with one wing showing a blister at its base; some females, also, have the not blistered wing singed, and one female looks like dumpy and blistered. In  $F_2$  we find  $\pm$  slender flies, slender singed, slender blistered, soft blistered, and bran blistered, the last-named two classes merging into each other. The expectations are:

$$\begin{aligned} &\text{Cross } +, -; \text{svr}^{\text{poi } s} / \text{svr}^{\text{poi } s} \times \text{bran}/\text{bran}; \text{svr}^{\text{poi } sq} \\ &F_1: \text{♀ } \text{♀ } \text{bran}/+, \text{svr}^{\text{poi } s} / \text{svr}^{\text{poi } sq}, \text{♂ } \text{♂ } \text{bran}/+, \text{svr}^{\text{poi } s} \\ &F_2: \text{♀ } \text{♀ } : 1 +/+, \text{svr}^{\text{poi } s} / \text{svr}^{\text{poi } s}, 1 +/- \text{svr}^{\text{poi } sq} / \text{svr}^{\text{poi } sq}, 2 \text{bran}/+; \\ &\quad \text{svr}^{\text{poi } s} / \text{svr}^{\text{poi } s}, 2 \text{bran}/+; \text{svr}^{\text{poi } sq} / \text{svr}^{\text{poi } sq}, 1 \text{bran}/\text{bran}; \text{svr}^{\text{poi } s} / \text{svr}^{\text{poi } s}, \\ &\quad 1 \text{bran}/\text{bran}; \text{svr}^{\text{poi } sq} / \text{svr}^{\text{poi } sq} \\ &F_2: \text{♂ } \text{♂ } \text{dto for second chromosome with } \text{svr}^{\text{poi } s} \text{ and } \text{svr}^{\text{poi } sq}, \text{ respectively.} \end{aligned}$$

The new-type slender blistered females in  $F_2$  might be the  $\text{bran}/+$  heterozygotes, either with homozygous soft or the compound  $\text{poi } s/\text{poi } sq$ . But the corresponding males must have the soft  $X$ , as the combination with square  $X$  is known to be pointed. The  $F_1$  males agree with this, but the  $F_1$  females also show the new type. We know, further, that  $\text{bran}/+, \text{svr}^{\text{poi } s}$  does not show the blistered type; from which it follows that the  $\text{bran}$  locus which is responsible for the  $\text{bran}$  blist type with basal blisters is also responsible for the slender blistered type with the same blisters, i.e., the  $\text{bran}$  is a different one, called  $\text{bran}^2$ . Further generations and backcrosses agree with this.

As table 60 shows, in  $F_1$   $\text{soft} \times \text{bran blist}$  a single female with short, dumpy, blistered wings appeared (6951). Ordinary  $F_2$  from this  $F_1$  (i.e., not from the unusual female) segregated (7094 ff) into slender,  $\text{poi } s$  blist, and sing and soft blistered with transitions to  $\text{bran}$  blistered. The cross was  $(+/+, \text{poi } s/\text{poi } s \times \text{bran}^2/\text{bran}^2, \text{poi } sq)^2$ . Therefore in  $F_2$   $\text{bran}^2/\text{bran}^2, \text{poi } s/\text{poi } s$  segregated as a new combination with the phenotype soft blistered and  $\text{bran}^2/\text{bran}^2, \text{poi } s/\text{poi } sq$  with a phenotype between soft blistered and  $\text{bran}$  blistered. But the unusual  $F_1$  ♀ dumpy blistered mated to a brother produced in  $F_2$  (7097), a segregation with three-fourths of the individuals as before, but with one-fourth divided into soft blistered and the grand-maternal type. If we make the hypothesis that the new type was based upon a new mutant  $\text{bran}^{ab}$  in conjunction with the pointed allele  $\text{poi } s$  or  $\text{poi } sq$ , the cross was  $\text{bran}^{ab}/+, \text{poi } s/\text{poi } sq \times \text{bran}^2/+, \text{poi } s$ . This segregated one-fourth  $\text{bran}^{ab}/\text{bran}^2$ ;  $\text{poi } s/\text{poi } s$  or  $\text{poi } s/\text{poi } sq$  ♀♀ and  $\text{poi } s$  or  $\text{poi } sq$  ♂♂ one of which was soft blist, the other dumpy blist. As  $\text{poi } sq$  is known to tend to produce a square or dumpoid phenotype, we assume that the new type was  $\text{bran}^{ab}/\text{bran}^2, \text{poi } sq$  ♂ or  $\text{poi } sq/\text{poi } s$  ♀. The original ♀ must have been heterozygous  $\text{bran}^{ab}/+$ , since no  $\text{bran}$  segregated in  $F_2$ , which would mean that  $\text{bran}^{ab}$  is dominant. But it behaved as a recessive in  $F_2$  and in all further crosses, and hence we must assume that the dominance in the first female, arisen by mutation, was an accidental feature. From extracted "dumpy blistered" from the  $F_2$  7097 a true breeding stock was established which in further tests turned out to be homozygous  $\text{bran}^{ab}/\text{bran}^{ab}, \text{poi } sq/\text{poi } sq$ . Immediately after isolation of this stock, the dumpy blistered flies were tested against second-chromosome markers, with the result that the two original right ends of the second chromosomes were needed for the phenotype. A backcross with  $\text{bran}$  blistered resulted in a nonclassifiable series of phenotypes from  $\text{dp blist}$  to  $\text{bran blist}$ , which is expected from the backcross  $\text{bran}^{ab}/\text{bran}^2, \text{poi } s/\text{poi } sq \times \text{bran}^2/\text{bran}^2, \text{poi } sq$ . Another

test backcross of dp blist  $\times$  soft blist  $F_1$  with soft blist = bran<sup>ab</sup>/bran<sup>2</sup>; poi s/poi sq  $\times$  bran/bran, poi gave the expected segregation into dumpy blist and soft blistered. The decisive test was made after the stock had become homozygous by breeding from typical dumpy blistered, i.e., bran<sup>ab</sup>/bran<sup>ab</sup>, poi sq/poi sq selected from bran<sup>ab</sup> bran<sup>2</sup>; poi s/poi sq inbred, (stock 7960 dp bl). Females of this homozygous stock  $\times$  bran  $\sigma$  produced in  $F_1$  all  $\text{♀♀}$  bran, all  $\text{♂♂}$  pale soft folded. The latter were a very characteristic new type. The wings are long and soft and blistered when hatching and later fold lengthwise into a wing looking like a wet towel. The paleness of the silver type is more extreme than usual. The  $\text{♀♀}$  bran are bran<sup>ab</sup>/bran, and the folded  $\text{♂♂}$  are bran<sup>ab</sup>/bran, poi sq.  $F_2$  shows a very characteristic segregation into 3  $\text{♀}$  and  $\text{♂}$  of the same pale soft folded type, including also soft blist, 4  $\text{♀}$  and  $\text{♂}$  bran, and less than 1 ( $1/8$ ) dumpy blistered (lower vitality). The segregation was clearly:

Cross: bran<sup>ab</sup>/bran, poi sq/+  $\times$  bran<sup>ab</sup>/bran, poi sq  
 $F_2$ :  $\text{♀}$  1: bran<sup>ab</sup>/bran<sup>ab</sup>, poi sq/poi sq = 1 dumpy blistered  
 2: bran<sup>ab</sup>/bran<sup>ab</sup>, poi sq/+ = 1 bran  
 3 and 4: bran<sup>ab</sup>/bran, poi sq/poi sq = 2 soft folded blistered  
 5 and 6: bran<sup>ab</sup>/bran; poi sq/+ = 2 bran  
 7: bran/bran, poi sq/poi sq = 1 soft blistered-dp blistered  
 8: bran/bran; poi sq/+ = 1 bran

The  $F_2$  types were tested in  $F_3$  and by outcrossing to parent stock. The constitution was confirmed, but it turned out that homozygous dp blist had a tendency toward the heterozygous soft blistered folded phenotype, possibly caused by external conditions.

The different phenotypes thus obtained are tabulated below (pp. 388 f.). Clearly, bran<sup>ab</sup> alone has the phenotype bran, but in combination with poi sq, dumpy blistered. The effect is somewhat dominant in compounds, but not in the heterozygote, though the first mutant female had shown dominance in the heterozygote.

Still another combination can be made by crossing poi blist = bran/bran; svr<sup>poi bl</sup> with bran blist = bran<sup>2</sup>/bran<sup>2</sup>, svr<sup>poi sq</sup>. The reciprocal  $F_1$  males are somewhat different.  $F_1$  poi bl  $\times$  bran bl, i.e.,  $\text{♂}$  bran/bran<sup>2</sup>, svr<sup>poi bl</sup>, are still blistered, more pointed, and only a few have short wings. The reciprocal cross with  $\text{♂}$  bran/bran<sup>2</sup>, svr<sup>poi sq</sup> does not show blistering in all individuals, which is clearly an influence of the svr allele; but many males possess short wings (like rudimentary). In both reciprocal  $F_2$  one-eighth of the males have the rudimentary type, which then must be the combination bran/bran, svr<sup>poi sq</sup> or bran<sup>2</sup>/bran<sup>2</sup>, svr<sup>poi bl</sup>.

As the X chromosomes of the first found allele poi s which was used for the further checks had been derived either from wild type or bs stocks (or both), one might not expect the presence of the different translocations and transpositions presumably derived from px bl (though svr<sup>poi bl</sup> was derived also from wild type!). If they were present, it would mean that the mutation to the soft allele was somehow linked up with these other changes, as it had also to be assumed for svr<sup>poi bl</sup>. Therefore the sex ratios of outcrosses involving both poi alleles were checked. They are as shown in table 61, both for soft derived from a px bl X, and for soft in slender derived from a foreign X.

Table 61 shows a definite rule: High ratios for slender, either near 4:3 or 2:1, are always present when the father introduces a not sl X chromosome and the

mother is homozygous or heterozygous for sl, i.e., all crosses (sl  $\times$  N)  $\times$  N, sl  $\times$  (N  $\times$  sl). Normal sex ratios obtain whenever the father is slender. This looks as if one-half of both ♀♀ and ♂♂ homo- or hemizygous slender were lethal in the presence of homo-

TABLE 61  
SEX RATIOS INVOLVING svr<sup>poi</sup>

Cross	♀	♂	Ratio
slender (sl) inbred.....	614	518	1.18
RF <sub>2</sub> sl $\times$ (+ $\times$ sl).....	751	560	1.32
RF <sub>2</sub> sl $\times$ (sl $\times$ +).....	430	393	1.09
RF <sub>2</sub> (sl $\times$ +) $\times$ sl.....	652	611	1.07
RF <sub>2</sub> (+ $\times$ sl) $\times$ sl.....	434	429	1.01
RF <sub>2</sub> $\bar{y}$ $\times$ ( $\bar{y}$ $\times$ sl).....	881	838	1.05
RF <sub>2</sub> (sl $\times$ Xple) $\times$ Xple.....	241	132	1.83
RF <sub>2</sub> sl $\times$ (SD $\times$ sl).....	145	74	2.0
F <sub>1</sub> sl $\times$ bran.....	81	42	2.0
F <sub>1</sub> sl $\times$ a px sp.....	45	25	1.8
F <sub>1</sub> sl $\times$ soft.....	68	53	1.3
F <sub>1</sub> soft $\times$ bran.....	324	189	1.71
F <sub>1</sub> soft $\times$ bran blist.....	31	28	1.1
F <sub>2</sub> (soft $\times$ bran) <sup>2</sup> .....	159	175	0.9
F <sub>2</sub> (soft $\times$ bran blist) <sup>2</sup> .....	404	318	1.27
F <sub>2</sub> (sl $\times$ bran) <sup>2</sup> .....	185	154	1.2
RF <sub>2</sub> (sl $\times$ a px sp) $\times$ a px sp.....	92	69	1.32

zygous autosomes, just as was noted for the other poi alleles. (This does not hold for bran, which is not foreign!) A check with marked second chromosomes actually reveals the same situation as was found for poi: most males homozygous for a for-

TABLE 62  
sl BACKCROSSES WITH ll sp AND a px sp  
(bran in slender remains heterozygous and is therefore without influence)

Cross	♀				♂					
	+	ll sp	ll	sp	+	ll sp	ll (sp) sl	sp	ll	sl
1. (ll sp $\times$ sl) $\times$ sp.....	320	243	1	2	192	116	..	2	..	95
Expect.....	1	1	c.o.	c.o.	1	1	1	c.o.	c.o.	1
	+	a px sp	a px	sp	+	a px sp	a px (sp), sl	a px	sp	sl
2. (sl $\times$ a px sp) $\times$ a px sp..	48	41	2	1	26	25	4	2	..	12

Sex ratio: (1) 1.4; (2) 1.33.

eign second chromosome are lethal (table 62). The result is exactly as with the pointed alleles. Soft suppresses sp. In the first cross the group ll (sp) sl can therefore hardly be distinguished (except for the pale color). This combination is almost lethal but for a few survivors, visible in the second cross. Moreover, the slender class is deficient. We point to the discussion of the parallel facts for pointed. Also, the

transposition in the X chromosome was tested, though only on a small scale, with results paralleling those for pointed. All these facts are of importance for the discussion of the origin of these mutants.

Finally, the standard translocation test, which in some alleles showed a suppressor action with eyeless, is presented in table 63. The females show clearly the suppressor action as seen when plus and ey, e and e ey, bw and bw ey are added (see also table 55). But in the males this is not the case, suggesting that the interaction between autosomes and the X chromosome is not a simple one (see discussion above, p. 359).

*The alleles bran<sup>dp</sup> (bran dumpy) and bran<sup>r</sup> (bran rudimentary).*—The very remarkable allele bran<sup>dp</sup> has appeared repeatedly. As it has no visible effect except in combination with one of the svr<sup>poi</sup> alleles, it was recognized only where the latter was present. The combination of bran<sup>dp</sup> with poi has a highly variable effect. The majority of the individuals resemble pointed; a certain percentage have one pointed and

TABLE 63  
Patt × (Patt × slender)

♀								♂							
Patt	bw e	bw ey	e ey	bw	e	ey	+	bweey	bw e	bw ey	e ey	bw	e	ey	+
91	102	57	95	172	123	76	165	90	44	117	77	129	79	111	191

one dumpy (truncated) wing; some have one dumpy wing and one with all transitions from pointed to dumpy, and another group have both wings truncated; further variants depend upon the respective poi allele, as will be described below. (See text fig. 2.) Thus, bran<sup>dp</sup> can only be discovered as flies with one wing poi, one truncated (abbreviated henceforth poi: dp), or both truncated in an otherwise pointed culture. As such, they were repeatedly found in svr<sup>poi</sup> stock as single flies and also in crosses with this stock. The following are some of the exactly known pedigrees.

1. We reported above on one of the crosses of pointed blistered × Bld T (3922) which produced in F<sub>1</sub> pointed males and Bld females, all looking like Bld (1) Df. F<sub>2</sub> (4539) produced both Bld females and males of this type. It turned out that a dominant Bran allele had arisen which, heterozygous with Bld, produced these Minute-like blond broad-winged flies (see above, p. 363). From this F<sub>2</sub> the segregating poi blist flies (= bran/bran, poi<sup>b1</sup>) were extracted. Their offspring (F<sub>3</sub> 4713) consisted of only 25 ♀ 6 ♂ poi blist, 1 ♀ 1 ♂ + and 1 ♀ truncated and blistered. As rudimentary was one of the mutants derived before, this ♀ dp blist was crossed to a male containing both pointed and rudimentary in the X chromosome. All offspring (no. 5,000) were pointed as expected, but 1 ♀ was poi: dp. We remember that the great-grandparents had contained dominant Bran, which in the presence of poi produces soft blistered flies. The offspring of this F<sub>3</sub> may then contain rudimentary, pointed and soft blistered, and in addition poi: dp or dumpy (dp not meaning the locus dp, but only the phenotype of this description) if it is inherited. The following generations up to the establishment of the stock called pointed dumpy (poi: dp), being the new bran<sup>dp</sup> + poi, are recorded in table 64 (note that r contains poi but is epistatic).

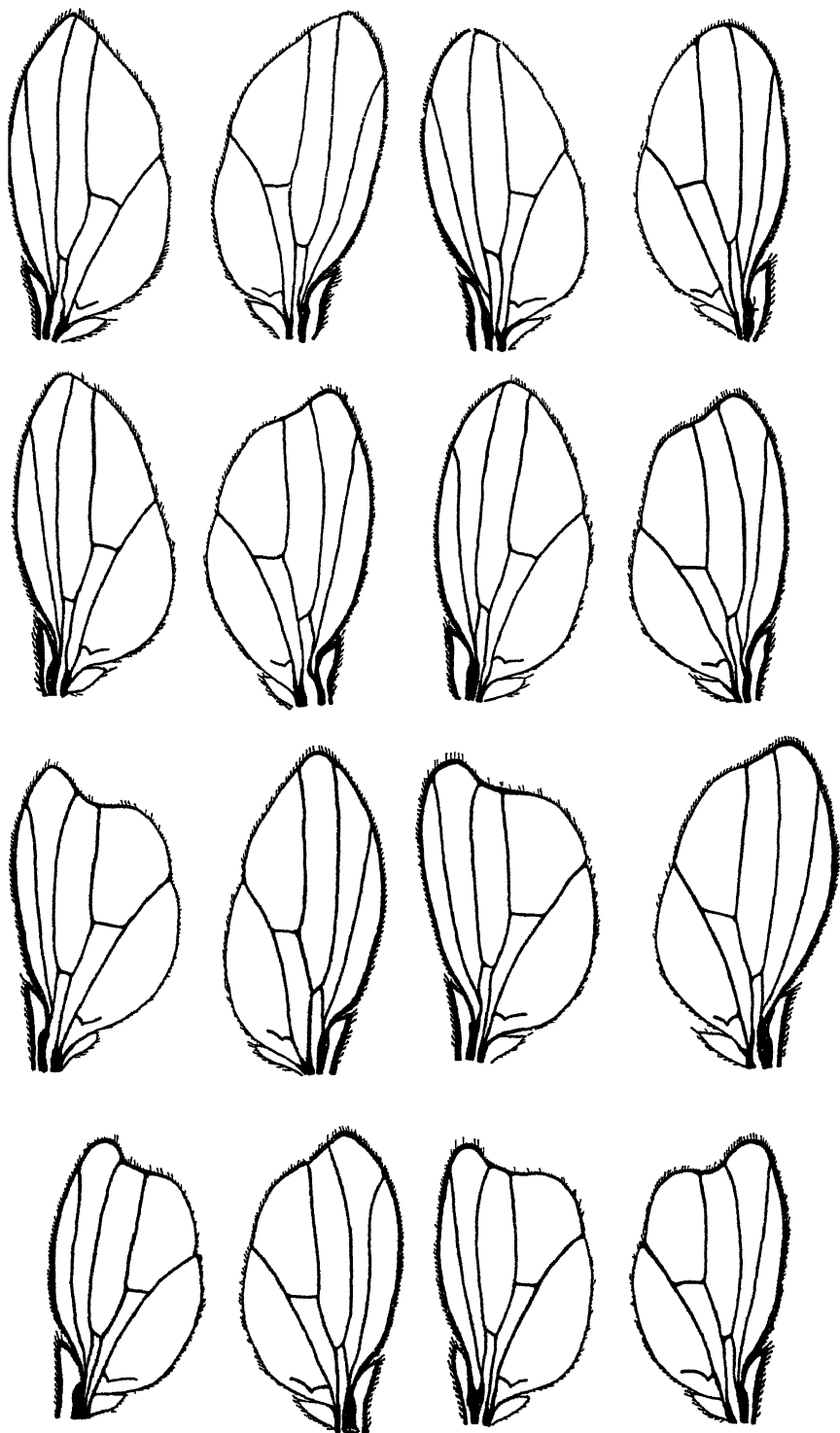


Fig. 2. Series of wing phenotypes of *poi : dp*. The beginning, both wings pointed, omitted.  
Both wings of one fly are always drawn.

TABLE 64  
PEDIGREE OF poi:dp AFTER F<sub>8</sub> 5000

No.	Cross	♀						♂						Remarks
		poi	poi:dp	dp	soft bl	rud	N-Df	dp bl	poi	poi:dp	dp	soft bl	rud	
5101	F <sub>8</sub> 5000 <sup>3</sup> ♀ poi:dp...	45	8	13	8	..	..	..	21	..	..	..	15	..
5102	F <sub>8</sub> 5000 <sup>2</sup> ♀ poi.....	53	7	5	12	..	..	..	19	..	..	..	22	..
5103	F <sub>8</sub> 5000 <sup>3</sup> ♀ poi.....	66	1	5	16	..	..	..	35	..	..	15	38	..
5166	F <sub>8</sub> 5101 <sup>2</sup> poi:dp × poi.....	54	3	13	14	..	..	..	19	2	12	12	14	..
5171	F <sub>8</sub> 5101 <sup>2</sup> soft bl × r	12	1	9	8	..	..	..	14	..	..	24	22	..
5172	F <sub>8</sub> 5101 <sup>2</sup> poi × r...	43	2	..	19	39	..	..	27	..	..	11	..	rud. contains also dp, cannot be classified
5208	F <sub>8</sub> 5166 <sup>3</sup> poi × dp...	56	3	2	..	..	..	..	32	..	..	..	..	..
5209	F <sub>8</sub> 5166 <sup>3</sup> poi × soft bl.....	19	..	..	11	8	..	..	8	..	..	7	16	1 gynander ♀ poi ♂ dp
5204	F <sub>8</sub> 5208 <sup>3</sup> poi:dp × poi.....	64	21	5	..	..	..	..	33	..	..	..	23	10 ♂ dwarf
5337	F <sub>8</sub> 5204 <sup>3</sup> poi:dp × poi.....	46	3	4	..	..	..	..	48	..	6	..	22	1 ♂ bl 2 ♂ dwarf
5328	F <sub>8</sub> 5204 <sup>3</sup> poi × rud.	44	..	..	..	19	..	..	38	..	..	..	..	Some N ♀ show dp
5329	F <sub>8</sub> 5204 <sup>3</sup> poi × poi.	76	..	..	..	..	2	..	50	..	..	..	..	Some N ♀ show dp
5357	F <sub>8</sub> 5329 <sup>3</sup> N ♀ ♂ ?	114	6	7	..	..	54	..	55	..	..	10	..	24 ♀ are rud bl
5373	F <sub>8</sub> 5357 <sup>3</sup> ♀ poi.....	57	6	6	..	..	..	..	55	4	..	..	..	Stock poi:dp
5374	F <sub>10</sub> 5357 <sup>3</sup> ♀ poi.....	75	4	..	..	..	..	..	87	..	..	..	..	Some N ♀ show dp
5375	F <sub>10</sub> 5357 <sup>3</sup> ♀ N-Df	34	1	1	..	..	27	..	34	1	..	..	..	Some N ♀ show dp
5376	F <sub>10</sub> 5357 <sup>3</sup> ♀ N-Df	34	1	1	..	..	47	..	33	..	..	..	..	24 ♀ are rud bl
5378	F <sub>10</sub> 5357 <sup>3</sup> ♀ dp	55	12	4	..	24	..	24	59	1	..	3	..	Stock poi:dp
5390	F <sub>11</sub> 5373 <sup>3</sup> poi:dp...	60	8	13	..	..	..	..	55	7	..	..	..	♀ most rud bl, few soft bl; ♂ most soft bl, few rud bl
5391	F <sub>11</sub> 5373 <sup>3</sup> dp × poi:dp.....	30	8	12	..	..	..	8	25	4	1	..	..	..
5393	F <sub>11</sub> 5373 <sup>3</sup> rud bl × soft bl.....	..	..	..	..	All	..	..	..	..	..	All	..	..

The table shows how the *poi:dp* stock with its typical variation *poi*→*poi:dp*→*dp*→*dp bl* was extracted from the offspring of the original *poi:dp* flies. There is nothing unexpected otherwise. The rudimentary X chromosome was again selected out. As there is a very high percentage of crossing over between *svr<sup>poi</sup>* and *r* introduced into the same chromosome, it cannot be decided whether finally the allele *svr<sup>poi</sup>* or *svr<sup>poi bl</sup>* went into the *poi:dp* stock. The dominant Bran originally present was also selected out. It produces in different combinations with *poi* alleles rudimentary blistered and soft blistered phenotypes as selected in no. 5393. This is important, because as we shall later see, the stock *poi:dp* sometimes contains ordinary bran. This must be the result of mutation after the stock was isolated. The new mutant Notch Deficiency (since 5329 F<sub>8</sub>) can show pointed, *poi:dp*, and *dp* phenotypes and is therefore independent of the *poi:dp* genotype.

TABLE 65  
PHENOTYPE OF BROODS FROM PAIRS FROM *poi:dp* STOCK

No.	Parental phenotype	♀				♂			
		<i>poi</i>	<i>poi:dp</i>	<i>dp</i>	<i>dp bl</i>	<i>poi</i>	<i>poi:dp</i>	<i>dp</i>	<i>dp bl</i>
I	<i>poi</i> × <i>poi</i> .....	19	4	1	..	12	..	3	..
II	<i>dp</i> × <i>dp</i> .....	15	6	16	..	15	6	11	..
III	<i>dp</i> × <i>dp</i> .....	71	20	13	8	73	4	10	4
IV	<i>dp</i> × <i>dp</i> .....	35	15	31	9	53	2	4	10
7113	<i>poi:dp</i> × <i>poi:dp</i> .....	2	11	6	..	15	1	..	..

2. Thirty F<sub>2</sub> crosses of *svr<sup>poi</sup>* × *svr<sup>poi bl</sup>* were bred. Among the 6,000-odd flies were 2 ♀ with *dp* wings. Only one gave offspring which were normal, as were two more generations. Again, in one F<sub>3</sub> from dwarfed F<sub>2</sub> parents 1 ♀ *poi:dp* among 14 ♀ was found. She produced only 6 daughters, 1 again *poi:dp*, which was sterile.

3. In a pointed stock containing a bobbed allele which produces etching of the abdominal tergites (see below, p. 390), called *poi achi* 6317, two females *poi:dp* (also *achi*) were found. They were sterile. Simultaneously, one male with scalloped wings appeared which turned out to be a *Beadex* allele. This ♂ mated to a *poi achi* sister produced only 3 daughters, 1 ♀ *poi bb achi*, 1 ♀ *dp bb achi*, 1 ♀ *bb achi* with a new type of wing. The dumpy-like ♀ crossed with the *poi:dp* stock produced no *poi:dp* in F<sub>1</sub> or F<sub>2</sub>, but soft blistered in F<sub>3</sub>. In later generations the dumpy blistered, rudimentary blistered, and soft blistered flies segregated together. This prevents a decision on the composition of the original dumpy ♀ which might have contained *bran<sup>ab</sup>* or *bran*.

4. We shall report below that *poi:dp* has also been produced by X-raying and, in addition, that single individuals have cropped up in different crosses so frequently that they were not tested any more.

*The phenotype.*—The stock *poi:dp* always contains a majority of pointed flies and a minority of *poi:dp* and *dp* phenotypes. But in breeding from pairs rather constant ratios are obtained, as table 65 shows. As a rule the largest class has pointed wings. Flies with one wing pointed and the other like dumpy, and flies with both wings truncated, of equal or unequal length (all transitions), occur in about equal numbers, and in large broods—they are usually small—dumpy phenotypes

with a blister in one or both wings are sometimes found (see below, allele *bran*). Females and males contain the same types, but the aberrant type has a lower expressivity in males (as most of the wing types studied in this paper do), visible as a lower percentage of the not pointed types. (All flies are pale, being homozygous *svr* alleles.)

Frequent selections of the types were made. One experiment over a few generations is presented in table 66. In the first sections of the table, pointed flies were

TABLE 66  
SELECTION FOR pointed IN *poi:dp*, ALWAYS TWO BROODS ADDED

Generation	♀ phenotype				♂ phenotype			
	<i>poi</i>	<i>poi:dp</i>	<i>dp</i>	<i>dp blist</i>	<i>poi</i>	<i>poi:dp</i>	<i>dp</i>	<i>dp blist</i>
1.....	68	12	6	..	65	2	..	..
2.....	91	3	1	..	65	..	..	..
3.....	79	20	13	4	105	3	..	..
4.....	76	4	..	1	105	4	..	..
5.....	47	2	..	..	32	..	..	..

SELECTION FOR THE PHENOTYPE *poi:dp*

	♀				♂			
1.....	28	3	5	..	30	5	1	..
2.....	29	..	..	1	36	1	..	..
3.....	30	1	4	2	32	2	..	2
4.....	28	5	10	2	29	4	6	..
5.....	24	3	4	1	26	3	3	..

SELECTION OF *dp* FROM STOCK

	♀				♂			
	35	3	..	..	29	..	..	..
	21	1	..	..	26	..	..	..

selected from the *poi:dp* stock and each subsequent generation. One might say that a little effect was observed, probably by selection of modifiers, though the variation in consecutive generations does not seem to show a definite trend. In the same way, *poi:dp* was selected without any visible effect. Neither did dumpy selected from stock increase its share. But the few *dp blist* individuals coming out occasionally and also found in the stock always bred true (see below). As it appeared that relatively more truncated flies appear in mass cultures, probably external conditions are also involved. It did not seem worth while to follow up this subject.

*Genetics.*—All tests for the pointed allele in *poi:dp* show that it is the ordinary *svr<sup>poi</sup>* (see discussion of origin) and that it is always present in homozygous condition in all the phenotypes described. Also, the other features of the *svr<sup>poi</sup>* stock are found as seen in the sex ratios of crosses, which thus do not need further discussion. A standard test with dominant markers revealed that *poi:dp* (*all phenotypes*) requires the simultaneous presence of *svr<sup>poi</sup>* homo- or hemizygous and something in



the second chromosome of the *poi:dp* stock, also in homozygous condition, as table 67 shows.

The father was heterozygous for all autosomes and had the *poi X* chromosome. Flies heterozygous for *S* (or *SD*) introduced by the father without crossing over were all pointed; flies without *S*, i.e., homozygous for the second chromosome from *poi:dp*, showed the typical series from *poi* to *poi:dp*, *dp*, and *dp blist* in both sexes whether *D* was present or not.

This result suggested an interaction with a *bran* allele, especially after the reciprocal cross with crossing over showed a location of the second chromosome locus far from *Star*. All other tests, such as  $\underline{y} \times \textit{poi:dp}$ , and following generations or crosses with recessive markers agreed that the variable *poi:dp* types were the product of collaboration between *svr<sup>poi</sup>* and a homozygous second-chromosome locus.

TABLE 67  
♀ 5390 *poi:dp* × ( $\underline{y}$ , *S/+*, *D/+* × *poi:dp*)

Phenotype ♀									
<i>S</i>	<i>S, D</i>	<i>D, poi</i>	<i>D, poi:dp</i>	<i>D, dp</i>	<i>D, dp bl</i>	<i>poi</i>	<i>poi:dp</i>	<i>dp</i>	<i>dp bl</i>
38	68	34	2	10	12	21	1	12	12
Phenotype ♂									
<i>S</i>	<i>S, D</i>	<i>D, poi</i>	<i>D, poi:dp</i>	<i>D, dp</i>	<i>D, dp bl</i>	<i>poi</i>	<i>poi:dp</i>	<i>dp</i>	<i>dp bl</i>
44	51	35	1	5	10	19	3	1	11

When the second chromosome was tested for *bran*, it turned out that the *poi:dp* stock contained in an irregular way another *bran* allele with phenotypical *bran* effect which, however, has nothing to do with the *poi:dp* phenotypes—a fact which led to much confusion until analyzed. The majority of crosses *poi:dp* (all types except that which was called above *dp blist*) × *bran* produce normal daughters and pointed sons. Anticipating that *poi:dp* contains an allele *bran<sup>ap</sup>*, this means that the combination ♀ *bran<sup>ap</sup>/bran*, *svr<sup>poi</sup>/plus* is normal and the ♂ with the same *bran* compound and *svr<sup>poi</sup>* is pointed, i.e., *bran<sup>ap</sup>* acts like wild type. But in about one-fourth of such crosses *F*<sub>1</sub> females are half normal half *bran*, and the majority of males pointed, a minority soft blistered, or rudimentary-like and blistered. Soft blistered, when tested, turned out to be the ordinary *bran/bran*, *svr<sup>poi</sup>* combination except for a tendency toward dumpy blistered and the *rud blist* (=phenotype rudimentary blistered) types. The mother, therefore, had been heterozygous for *bran* or an allele which, as stated above, must have originated as a mutant in the *poi:dp* stock after its isolation and spread through the stock. If this is true, occasional matings of heterozygous *bran* flies must occur in the stock, resulting in ¼ soft blistered and *rud blist* offspring. As this type has a lower viability, it cannot hold its own and will remain rare. Actually, occasional soft blistered or rudimentary blistered (phenotype, not locus *r*!) flies appear in the *poi:dp* stock. Their analysis shows that they are homozygous for a *bran* allele, different from *bran<sup>ap</sup>*, and, in addition, for pointed. The frequency, sometimes preponderance, of the rudimentary blistered type in this combination (which is rare in ordinary soft *blist*) indicates

that another allele than bran is involved, which we shall call bran<sup>r</sup>. This has turned out to be true, as the compounds and combinations show: bran/bran = broad round, bran<sup>dp</sup>/bran<sup>dp</sup> = +, bran<sup>dp</sup>/bran<sup>r</sup> = broad round tendency to dumpy, bran, bran; poi = soft blistered, bran<sup>dp</sup>/bran<sup>dp</sup>; poi = pointed to poi:dp to dp; bran<sup>dp</sup>/bran<sup>r</sup>; poi = pointed, bran<sup>dp</sup>/bran<sup>r</sup>; poi = the poi:dp series, bran<sup>r</sup>/bran<sup>r</sup>; poi = rud blist-soft blist (more rud blist) bran<sup>r</sup>/bran; poi = rud blist-soft blist (more soft blist).

We have already seen in the selection experiments (see p. 377) that the type dp blist (varying into rud and soft blist) sometimes segregated from poi:dp and, if

TABLE 68  
F<sub>1</sub> CROSSES WITH DIFFERENT TYPES FROM poi:dp STOCK

No.	Type of poi:dp parent	Other parent	F <sub>1</sub> ♀	F <sub>1</sub> ♂	
I	poi:dp ♀ . . . .	bran	+	poi	A later repetition with 10 pairs gave three times, this result; seven times, result like IV
II	dp ♀ . . . . .	bran	+	poi	Sex ratio, 2 : 1
III	poi ♀ . . . .	bran	+	poi	Sex ratio, 2 : 1
V	poi ♀ . . . . .	bran	+	poi	Sex ratio, 2 : 1
VI	dp ♀ . . . . .	bran	+	poi	Sex ratio, 2 : 1
IX etc.	poi:dp ♀ . . . .	+	+	poi	Fathers of different crosses are + or marked stocks
IV	dp ♀ . . . . .	bran	$\frac{1}{2}$ + $\frac{1}{2}$ bran	poi and soft blist	♂ rud blist and soft blist poorly viable, less than $\frac{1}{2}$
VII	dp ♀	bran	$\frac{1}{2}$ + $\frac{1}{2}$ bran	poi and soft blist	As above. Sex ratio, 6.5 : 1 (♀ 35 + 30) bran ♂ 8 poi 2 soft blist
VIII	poi:dp ♀ . . . .	poi	poi	poi	
XXV	poi:dp ♂ . . . .	$\underline{y}$ ♀	$\underline{y}$	poi	
7202	poi ♂ . . . . .	bran ♀	+	+	
7203	dp ♂ . . . . .	bran ♀	$\frac{1}{2}$ + $\frac{1}{2}$ bran	$\frac{1}{2}$ + $\frac{1}{2}$ bran	

extracted, bred true. This was bran<sup>r</sup>/bran<sup>r</sup>, poi. The numbers were always far below the expected  $\frac{1}{4}$ , owing to lower viability. Also the extracted line contained a rather small number of flies. Most of the data are self-explanatory and show the correctness of the formulations. The tables show, further, that in the first series made (table 68) the poi : dp phenotype did not contain bran<sup>r</sup>, but that the dp type did. In a later check (table 68, I, note) both conditions were found among 10 poi : dp types which were tested. The bran<sup>r</sup> combinations were frequently less viable. In one case (table 70, note d) it seems that bran<sup>r</sup> had just arisen as a mutation. In table 71 we analyzed the soft blist and rud blist types found occasionally in poi : dp stock as the combination dp bl-soft bl, i.e., bran<sup>r</sup>/bran<sup>r</sup>, poi. The results of the analysis agree with the expectations derived from the formulas. The low viability of this combination is also apparent.

Tables 68-71 contain some of the data from which the foregoing statements were derived.

An interesting check upon the analysis is derived from crosses with bran blistered stock which exhibits the phenotype of broad square wings with a basal blister based

upon the genotype  $\text{bran}^a/\text{bran}^a$ ,  $\text{poi}^a$ . A series of crosses between  $\text{poi:dp}$  and  $\text{bran}^a$  blist gave different  $F_1$  results, depending upon the presence or absence of  $\text{bran}^a$ , just as was the case with  $\text{poi:dp} \times \text{bran}$ . Table 72 contains the data arranged in genetical groups.

The cross is supposed to be:  $\text{bran}^{ap}/\text{bran}^{ap}$ ,  $\text{poi}/\text{poi} \times \text{bran}^a/\text{bran}^a$ ,  $\text{poi}^a$ . All daughters are  $\text{bran}^{ap}/\text{bran}^a$ ,  $\text{poi}/\text{poi}^a$ ; all sons,  $\text{bran}^{ap}/\text{bran}^a$ ,  $\text{poi}$ . The result is almost like that from inbreeding  $\text{poi:dp}$ , except that the percentage of  $\text{poi:dp}$

TABLE 69  
SOME  $F_2$  FROM CROSSES OF TABLE 68

No.	Cross	♀					
		+	poi	poi:dp	dp	bran	soft bl
I	( $\text{poi:dp} \times \text{bran}$ ) $I \times \text{bran}$ .....	50	..	..	..	38	..
II	( $\text{poi:dp} \times \text{bran}$ ) $I \times \text{poi:dp}$ .....	38	20	1	..	11	5
7699	( $\text{poi} \times \text{bran}$ ) <sup>2</sup> .....	11	13	..	..	2	1
7410	( $\text{bran} \times \text{poi}$ ) <sup>2</sup> 7202 <sup>2</sup> ..... (ratio)	3	..	..	..	1	..
7411	7203 <sup>2</sup> ( $\text{bran} \times \text{dp}$ ) <sup>2</sup> $F_1 +$ ..... (ratio)	3	..	..	..	1	..
7430-31	( $\overline{Y} \times 7045 \text{ poi:dp}$ ) <sup>2</sup> .....	All	..	..	..	..	..
7432	( $\overline{Y} \times 7044 \text{ soft bl}$ ) <sup>2</sup> ..... (ratio)	3 $\overline{Y}$	..	..	..	1 $\overline{Y}$	..
VII	( $\text{poi:dp} \times +$ ) $\times +$ .....	85	..	..	..	..	..
V	( $\text{poi:dp} \times +$ ) $\times \text{poi:dp}$ .....	35	19	2	10	..	..

No.	Cross	♂					
		+	poi	poi:dp	dp	bran	soft bl
I	( $\text{poi:dp} \times \text{bran}$ ) $I \times \text{bran}$ .....	29	44	..	..	23	..
II	( $\text{poi:dp} \times \text{bran}$ ) $I \times \text{poi:dp}$ .....	27	25	..	1	15	..
7699	( $\text{poi}^a \times \text{bran}$ ) <sup>2</sup> .....	15	7	..	..	3	3
7410	( $\text{bran} \times \text{poi}$ ) <sup>2</sup> 7202 <sup>2</sup> ..... (ratio)	3	3	..	..	1	1
7411	7203 <sup>2</sup> ( $\text{bran} \times \text{dp}$ ) <sup>2</sup> $F_1 +$ ..... (ratio)	3	3	..	..	1	1
7430-31	( $\overline{Y} \times 7045 \text{ poi:dp}$ ) <sup>2</sup> .....	All	..	..	..	..	..
7432	( $\overline{Y} \times 7044 \text{ soft bl}$ ) <sup>2</sup> ..... (ratio)	..	3	..	..	..	1
VII	( $\text{poi:dp} \times +$ ) $\times +$ .....	45	29	..	..	..	..
V	( $\text{poi:dp} \times +$ ) $\times \text{poi:dp}$ .....	22	20	1	..	..	..

<sup>a</sup> poi = phenotype in  $\text{poi:dp}$  stock.

and  $\text{dp}$  flies is much smaller, and almost nil in the males. This shows that  $\text{bran}^{ap}$  and  $\text{bran}^a$  are much more similar than are  $\text{bran}^{ap}$  and  $\text{bran}$ , and the compound action more or less intermediate. Actually, both tend to produce a truncated wing. As the former data indicate, the second group clearly had a mother heterozygous for  $\text{bran}^{ap}/\text{bran}^a$ . The cross thus was  $\text{bran}^{ap}/\text{bran}^a$ ;  $\text{poi}/\text{poi} \times \text{bran}^a/\text{bran}^a$ ;  $\text{poi}^a$ , resulting in

1)  $\frac{1}{2}$  ♀  $\text{bran}^{ap}/\text{bran}^a \text{ poi}/\text{poi}^a$

2)  $\frac{1}{2}$  ♀  $\text{bran}^a/\text{bran}^a \text{ poi}/\text{poi}^a$

3)  $\frac{1}{2}$  ♂ ditto with poi

4)  $\frac{1}{2}$  ♂ ditto with poi

Actually the ♀♀ and ♂♂ nos. 1 and 3 are the same as in the former group. i.e., pointed, with a small percentage of  $\text{poi:dp}$  and  $\text{dp}$ . The ♀♀ of no. 2 ought to be between dumpy (the  $\text{bran}^a$  type) and  $\text{bran}$  blist. Actually, the majority are dumpy, varying into a type of dumpy blistered and  $\text{bran}$  blistered. The males have the poi

TABLE 70

RF<sub>2</sub> AND F<sub>2</sub> FROM CROSSES F<sub>1</sub> poi:dp × bran Nos. 1-10 REPORTED IN NOTE TO No. 1, TABLE 68 (F<sub>1</sub> not segregating bran and soft blist, i.e., without bran<sup>a</sup> in poi:dp, is called normal, the others "segr.")

Cross	♀					
	F <sub>1</sub>	+	poi	bran	poi:dp types	dp blist-soft blist
1 <sup>2</sup> + × poi.....	norm.	42	38	7	..	..
10 <sup>2</sup> + × poi.....	segr.	41	157 <sup>a</sup>	6	..	2
9 <sup>2</sup> + × soft bl.....	<sup>b</sup>	2	..	1	..	1
10 <sup>2</sup> + × soft bl.....	segr.	2	..	1	..	1
		ratio		ratio		ratio
soft bl stock × soft bl no. 5.....	segr.	..	..	..	..	All
bran stock × soft bl from 9.....	segr.	..	..	All	..	..
bran stock × poi 9.....	<sup>b</sup>	½	..	½	..	..
soft bl stock × poi no. 1.....	norm.	..	½	..	..	½
♀ bran 10 × bran stock.....	segr.	..	..	All	..	..
poi:dp stock × soft bl 5.....	segr.	..	23 <sup>d</sup>	..	9	..
poi:dp stock × soft bl 6.....	segr.	..	13 <sup>d</sup>	..	8	10

Cross	♂				
	+	poi	bran	poi:dp types	± soft bl
1 <sup>2</sup> + × poi.....	20	16	5	..	..
10 <sup>2</sup> + × poi.....	16	26	14	..	..
9 <sup>2</sup> + × soft bl.....	15	2	14	..	9
10 <sup>2</sup> + × soft bl.....	2 <sup>c</sup>	..	1	..	1
	ratio		ratio		ratio
soft bl stock × soft bl no. 5.....	..	..	..	..	All
bran stock × soft bl from 9.....	..	..	All	..	..
bran stock × poi 9.....	½	..	½	..	..
soft bl stock × poi no. 1.....	..	½	..	..	½
♀ bran 10 × bran stock.....	..	..	½	..	½
poi:dp stock × soft bl 5.....	..	30	..	5	..
poi:dp stock × soft bl 6.....	..	16	..	4	13

<sup>a</sup> 1 ♀ new mutant (vortex).

<sup>b</sup> F<sub>1</sub> contained 109 ♀ +, 87 ♂ poi, 1 ♀ bran, 3 ♂ soft blist, i.e., 4 eggs out of 170 contained bran<sup>a</sup>, which looks like mutation.

<sup>c</sup> Considerable dominance of bran.

<sup>d</sup> 1 ♀ singed.

X chromosome which with bran<sup>a</sup> produces soft blistered, and therefore with the compound all transitions from dp blist to soft blist.

In one instance a strange result was obtained: all females were dumpy or dumpy blistered, all males dp blist or soft blist, with all transitions. The females were backcrossed both to bran blist and to poi:dp. The backcross (poi × bran bl) ♀ dp × bran blist gave:

14 ♀♀ dp, 9 dp bl, 14 ± soft bl (bran bl), 5 folded

1 ♂ dp, 4 dp bl, 40 bran bl-soft bl, 1 ♂ pointed singed

Comparing this with the results of the former group, it follows that the mother had been homozygous for bran<sup>a</sup> but exhibited phenotypically poi:dp instead of rudimentary blistered.

TABLE 71  
Soft bl from poi:dp Stock

No.	Cross	♀					Remarks
		poi	poi:dp	dp	dp bl	soft bl	
7001	♀ ♂ poi:dp from F <sub>1</sub> soft bl × poi.....	17	9	11	3	5	
7043	♀ ♂ poi from F <sub>1</sub> soft bl × poi.....	12	10	14	..	10	
7044	♀ ♂ dp from F <sub>1</sub> soft bl × poi.....	16	11	10	1	6	
	Σ.....	45	30	35	4	21	
7048	rud bl ♀, soft bl ♂ from poi:dp stock	..	1	1	..	All	Many rud bl Mut. N-def.
7198	7701 <sup>2</sup> poi:dp × poi.....	10	2	11	..	4	
7199	7701 <sup>2</sup> poi:dp × poi.....	22	5	6	..	8	
7200	7701 <sup>2</sup> ♀ ♂ poi.....	16	2	2	..	..	
7217	7701 <sup>2</sup> ♀ ♂ poi:dp.....	26	4	2	..	..	Only few rud bl
7227	7701 <sup>2</sup> ♀ ♂ poi.....	5	7	7	..	2	
7228	7701 <sup>2</sup> poi:dp × dp bl.....	..	4	7	..	3	
7249	7048 <sup>2</sup> soft bl.....	..	..	..	..	All	
7218	7048 <sup>2</sup> rud bl.....	..	..	..	..	All	Only few rud bl Part rud bl 10 rud bl 29 +, 23 bran Most rud bl
7219	7048 <sup>2</sup> rud bl × soft bl.....	..	..	..	..	All	
7223/24	7048 soft bl × stock soft bl and rec....	..	..	..	..	All	
7236	7044 rud bl ♂ × ♀ soft bl stock.....	..	..	..	..	21	
7260	7043 poi:dp × bran.....	..	..	..	1	..	Most rud bl
7439	7218 <sup>2</sup> rud bl.....	..	..	..	..	All	
7442	7227 <sup>2</sup> poi:dp.....	15	3	12	..	..	
7443	7227 <sup>2</sup> dp × poi:dp.....	10	3	7	..	..	
No.	Cross	♂					Remarks
		poi	poi:dp	dp	dp bl	soft bl	
7001	♀ ♂ poi:dp from F <sub>1</sub> soft bl × poi.....	27	8	3	6	5	
7043	♀ ♂ poi ditto.....	30	2	..	..	2	
7044	♀ ♂ dp ditto.....	17	..	1	1	5	
	Σ.....	74	10	4	7	12	
7048	rud bl ♀, soft bl ♂ from poi:dp stock	..	..	..	..	All	Most soft bl
7198	7701 <sup>2</sup> poi:dp × poi.....	5	2	..	..	3	
7199	7701 <sup>2</sup> .....	30	..	..	..	3	
7200	7701 <sup>2</sup> ♀ ♂ poi.....	19	..	..	..	..	
7217	7701 <sup>2</sup> ♀ ♂ poi:dp.....	13	1	1	..	..	Only few rud bl
7227	7701 <sup>2</sup> ♀ ♂ poi.....	12	6	2	..	2	
7228	7701 <sup>2</sup> poi:dp × dp bl.....	5	1	1	7	7	
7249	7043 <sup>2</sup> soft bl.....	..	..	..	..	All	
7218	7048 <sup>2</sup> rud bl.....	..	..	..	..	All	Only few rud bl
7219	7048 <sup>2</sup> rud bl × soft bl.....	..	..	..	..	All	
7223/24	7048 soft bl × stock soft bl and rec....	..	..	..	..	All	
7236	7044 rud bl ♂ × ♀ soft bl stock.....	..	..	..	..	13	
7260	7043 poi:dp × bran.....	7	..	..	..	6	Most rud bl
7439	7218 <sup>2</sup> rud bl.....	..	..	..	..	All	
7442	7227 <sup>2</sup> poi:dp.....	12	1	..	..	..	
7443	7227 <sup>2</sup> dp × poi:dp.....	14	1	1	..	..	

If there was no error in recording, no explanation can be offered. The backcross (poi : dp × bran bl) ♀ dp × poi : dp produced :

13 ♀♀ poi, 9 poi: dp, 11 dp, 2 dp bl, 14 ± soft blist

25 ♂♂ poi, 7 poi: dp, 7 dp, 11 ± soft blist

This is the expected result from the cross bran<sup>+</sup>/bran<sup>2</sup>, poi/poi<sup>++</sup> × bran<sup>40</sup>/bran<sup>40</sup>, poi under the same assumption regarding the grandmother as before (see table of phenotypes, p. 388).

Also, the first two groups of table 71 were tested in further generations and backcrosses as well as with testers like bran and soft blistered. The results were those expected from the foregoing data:  $\text{poi:dp}$  and bran blist could be extracted. In  $F_2$  ( $\text{bran}^{dp}/\text{bran}^s, \text{poi}/\text{poi}^{sq} \times \text{♂ ditto poi}$ )<sup>a</sup> the combination  $\text{bran}^{dp}/\text{bran}^{dp}, \text{poi}^{sq}$  could be obtained as a novelty, and in  $F_2$  ( $\text{bran}^{dp}/\text{bran}^s, \text{poi}/\text{poi}^{sq} \times \text{ditto } \text{♂ poi}$ )<sup>a</sup>

TABLE 72  
 $F_1 \text{ poi:dp} \times \text{bran blist}$

Mother (phenotype)	♀				♂				Remarks
	poi	poi:dp	dp	$\pm$ soft bl	poi	poi:dp	dp	$\pm$ soft bl	
5 times poi:dp. 4 times poi ..	296	14	22	..	237	1	1	..	1 ♀ ♂ poi bl 1 ♂ like soft bl (sterile)
5 times poi:dp	90	3	61	20	71	1	3	49	♀ soft bl = $\pm$ bran bl ♂ soft bl = $\pm$ soft bl
Once poi:dp ..	..	..	All	..	..	..	..	All	♀ dp and dp bl like bran bl ♂ dp bl and soft bl

the novelties  $\text{bran}^s/\text{bran}^s, \text{poi}^{sq}$  and  $\text{bran}^{dp}/\text{bran}^{dp}, \text{poi}^{sq}$  would segregate. In the first  $F_2$  the expectation is (see table of phenotypes below, table 74);

♀ 6 poi dumpy (=  $\text{poi} \rightarrow \text{poi} : \text{dp} \rightarrow \text{dp}$ , most poi), 2  $\pm$  soft blist

♂ 5 ditto (most poi), 1 bran blist, 1  $\pm$  soft blist, 1 novelty  $\text{bran}^{dp}/\text{bran}^{dp}, \text{poi}^{sq}$

In the second  $F_2$  the expectation is:

♀ 6 poi dumpy (most poi), 2 rud bl-soft blist

♂ 3 poi dumpy, 1 rud bl-soft bl, 2 nov.  $\text{bran}^{dp}/\text{bran}^s, \text{poi}^{sq}$ , 1 nov.  $\text{bran}^s/\text{bran}^s, \text{poi}^{sq}$ , 1 nov.  $\text{bran}^{dp}/\text{bran}^{dp}, \text{poi}^{sq}$

The segregation in the first case is shown in table 73. The ratios are not relevant,

TABLE 73  
 $(\text{poi:dp} \times \text{bran bl})^a$

	No. 7730	poi	poi:dp	All trans. dp-bran or rud bl
♀	7712 ff .....	94	14	74
♂	7712 ff .....	119	7	74

since the type dumpy belonging to the  $\text{poi:dp}$  range of variation is contained in the class with all transitions between dumpy, dumpy blistered, bran blist, and rud blist. This makes it difficult to ascribe a definite type to the combination  $\text{bran}^{dp}/\text{bran}^{dp}, \text{poi}^{sq}$ , but a few selection experiments point to a phenotype like dumpy blistered. We did not follow this up. The second type of  $F_2$  was still more difficult to analyze. We had only one  $F_2$  which was clearly in this category. Here the number of transitions between dumpy and soft blistered was very high, and the new combination  $\text{bran}^s/\text{bran}^s, \text{poi}^{sq}$  must have been in this group. Since in this case a majority of soft blistered types appeared, this is probably the phenotype of the combination.

It did not seem worth while to continue this analysis. Only a few combinations of  $poi:dp$  with slender, i.e.,  $bran^1/bran^1, poi^*$ , were made.  $F_1$   $poi:dp \times$  slender was slender in both sexes, with some singed flies, as is typical for slender crosses. Pointed dumpy crossed with soft ( $poi^*$ ) is pointed, with a few singed individuals. Thus  $bran^{ap}/+$  reacts with  $poi^*$ , as do the other  $bran$  alleles. By backcrossing to  $bran$  blist,  $\text{♀♀ } bran^{ap}/bran^*$ ,  $poi^*/poi$  may be obtained. Some of these are  $poi:dp$  and singed. The  $\text{♀♀}$  and  $\text{♂♂ } bran^1/bran^*$ ;  $poi^*/poi^*$  or  $poi$  are soft blist; a few are like  $bran$  blist (see table of phenotypes, table 74).

Pointed dumpy was assumed to be the product of  $bran^{ap}$  homozygous with  $svr^{po1}$ . To make sure that actually the ordinary  $svr^{po1}$  and no other allele or sex-linked modifier was involved, a  $svr^{po1}$  combined with white, i.e., practically the entire X chromosome right of  $svr^{po1}$ , replaced and marked with  $w$ , which is closely linked with  $svr^{po1}$ , was crossed to  $poi:dp$ . In  $F_2$  the  $poi:dp$  types appeared both with  $poi$  and with  $w$   $poi$ .

Finally, another peculiarity of the allele  $bran^{ap}$  must be mentioned. In crosses involving the locus ebony the heterozygote shows a dominance effect of ebony. We stated above that pointed itself tends to make ebony a little dominant (a trident visible). But the effect under discussion is different. It is clearly visible only in females heterozygous for both  $e$  and  $bran^{ap}$ . These females are actually sooty-like, with darkened body and wings. That  $bran^{ap}$  is responsible for this enhancing of dominance is proved by crossing a soft blistered fly from  $poi:dp$  stock, i.e.,  $bran^1/bran^1, poi$  with ebony; no dominance effect is obtained. In the  $F_2$  recombination  $bran^{ap}/bran^{ap}, e/e$  the ebony color seemed to be intensified; but since we did not succeed in extracting an intensified line this might have been a chance result.

One more remarkable feature is typical for the  $poi:dp$  stock. We discussed above the suppressors for eyeless found in the  $px$   $bl$  derivatives (see p. 359). At this occasion we mentioned a case in which eyeless seemed to be partially dominant in the combination  $bw/bw, e/e, ey/+, +$  meaning a fourth chromosome from  $poi$  stock. This dominance was restricted to the males of this combination alone. The same is typical for  $poi:dp$  as found in repeated checks at different times. In table 55 one such series is found. The numbers of the reciprocal classes with  $ey/ey$  and  $ey/+$  added together are about equal in the four groups (71, 64, 79, 125, the latter containing the more viable plus class); but there are only two  $bw$   $e$  males against 69  $bw$   $e$   $ey$  males. It must be supposed, therefore, that almost one-half of the  $bw$   $e$   $ey$  males are heterozygous for  $ey$ . The absence of the same effect in the three other groups is probably due to the simultaneous presence of the second- and (or) third-chromosome suppressors, discussed above. (It may be added that the same phenomenon was found in a  $poi$  allele, called  $sl:dp$ , produced by X rays, which in many respects parallels  $poi:dp$ . Its remarkable genetics will be presented separately. The Patterson backcross has been entered in table 55.)

In order to test the explanation involving a shift of dominance, produced either by something in the fourth chromosome of  $poi:dp$  (Dubinin effect?) or in the X chromosome of the same (the salivaries are normal), a considerable number of  $bw$   $e$   $ey$  males from  $Patt \times (Patt \times poi:dp)$  were crossed with unrelated eyeless females, and other, different, combinations were made as controls. The results were very strange. Actually, one-half of the  $bw$   $e$   $ey$  males turned out to be heterozygous for eyeless. But now the dominance effect had disappeared, though with a  $y$ ,

bw, e, ey mother the heterozygotes for ey in  $F_2$  had otherwise all chromosomes from the Patterson stock, just as the father had them. In another generation bred from eyeless and not eyeless,  $F_2$  males showed again the first breeding true, the second segregating ey and not ey in equal numbers. No explanation can be offered at present for the disappearance of the ey dominance effect. But we remember that these stocks exhibit the phenomenon of so-called mutable genes. We hope to continue the analysis, which is not connected directly with the present work.

The pointed dumpy combination has been analyzed so extensively because it parallels Demerec's so-called unstable genes in *Drosophila virilis* and furnishes, I think, a simpler explanation for those facts. This part of the problem has been discussed elsewhere (Goldschmidt, 1943). I repeat here only the main points.

In a number of papers Demerec (see 1941) described genetic phenomena in *Drosophila virilis* which he interprets as the result of unstable, ever-mutating genes. The best analyzed case is that of alleles at the miniature (small wings) locus. The decisive facts are as follows. Among a series of mt alleles, two, called 3 and 5, showed the phenomenon in question. This means that the offspring consisted of mt individuals, mosaics, and normals, the last-named breeding true, the others not. A mosaic is a fly with more or less miniature patches on normal wings, or with one normal and one miniature wing (see below). Unstable mt appears in three well-defined "suballeles." Of these,  $mt^b$  is stable;  $mt^c$  produces only miniature and mosaic offspring; and  $mt^a$ , normal, miniature, and mosaic offspring. Demerec considers these facts to be the consequence, in  $mt^a$ , of instability of the mt gene both in somatic and in germinal cells, and, in  $mt^c$ , of instability only in somatic cells. From the size of the mosaic spots and the number of wild-type flies, it is concluded that the "mutability" begins rather late in development and that the mutation is always one from miniature to normal, both in somatic and in sex cells. Furthermore, a series of modifiers exists. One increases the number of normals (called germinal mutability), but only in the  $mt^a$  line, which always segregates normals. Another series of modifiers (called S genes) increases only the mosaic spots (i.e., is supposed to act only upon somatic mutability). Without these enhancers a small percentage of flies show mosaic spots, and these spots rarely extend to an entire wing. In the presence of the modifiers the percentage of mosaics is increased up to 95 per cent, and one of the S genes frequently produces flies with one normal and one miniature or mosaic wing. There is, further, a sexual difference in all these effects. Finally, the a, b, c forms may change into one another and in a reversible way.

The first question is whether the series of phenotypes from pointed to dumpy, which we may call, in brief, the poi:dp effect, can be assumed to be comparable to those described by Demerec. There is no doubt in regard to the unchanged wings, i.e., miniature in *virilis*, pointed in my case, or for the type with one wing plus, one mt in *virilis*, one pointed, one dumpy in my case. The decisive types are the mosaic types in *virilis* and the intermediates in my case. Demerec assumes that the mosaic spots of miniature cells are genetically miniature, and the normal wing parts are genetically normal by somatic mutation. If one thinks of such mosaics in a general way, comparing them with gynanders or mosaic spots analyzed by markers, one is led to such an interpretation. But in the case in question this is not conclusive. A miniature wing is essentially one in which cell growth after pupation is inhibited (see Dobzhansky, 1929; Goldschmidt, 1935; Waddington, 1940). If this inhibition has a narrow threshold condition so that it will act only partially in the presence of a certain genetic condition, a mosaic-like structure of the wing will appear in the varying and asymmetrical patches of miniature tissue, including also the normal and the +:mt wings. Actually, we know of no case of a real mosaic within a wing, but many cases in which such an asymmetrical threshold effect occurs (see my discussion of the nicking effect, in Goldschmidt, 1940, pp. 222 ff.). In my case the decision is between a pointed and a more or less truncated wing. As truncation is a phenomenon at the wing edge, no mosaic in a plane can become visible. The mosaic produced by the transgressive threshold action appears in the form of a wing edge showing all transitions from pointed to dumpy, more or less asymmetrically. Thus, I believe that also in the miniature case there is no necessity for the assumption that the normal wing cells are genetically different from the miniature cells. A threshold condition acting within narrow limits can produce the apparent mosaic within the genetically miniature cells.



The data in my case indicate clearly the reason for the variable effect. The  $\text{bran}^{\text{dp}}$  allele, interacting with the  $\text{svr}^{\text{poi}}$  locus, is responsible for the threshold condition. Other  $\text{bran}$  alleles have a truncating effect alone, and, together with  $\text{svr}^{\text{poi}}$ , produce wing types such as soft blistered, truncated blistered, rudimentary blistered, etc. But  $\text{bran}^{\text{dp}}$  is an allele which alone has no visible effect, but with  $\text{svr}^{\text{poi}}$  hardly succeeds in pushing the pointed wing over the threshold toward a dumpy wing. In other words, the  $\text{bran}$  allele acts near the level of epistasis for whole wings or parts of them. In our case the explanation is clear: (1) a combination of two interacting loci; (2) the presence of one allele affecting epistasis near the threshold level; (3) the developmental physiology of the wing. As the parallelism with miniature is practically complete (including also modifiers and sexual difference), I conclude that  $\text{mt}^{\text{e}}$  collaborates with another allele which, like  $\text{bran}^{\text{dp}}$ , has no visible action alone, but, if epistatic, pushes wing-cell development toward normal, just as  $\text{bran}^{\text{dp}}$  pushes it toward truncation, and further, that  $\text{mt}^{\text{e}}$  is not an  $\text{mt}$  allele but ordinary  $\text{mt}$  in the presence of the other locus, say  $e$ , which has an epistatic action near the threshold.

In my case the location of  $\text{bran}^{\text{dp}}$  in the arc region is clear because alleles with visible action exist. In Demerec's case the second locus  $e$  is difficult to locate because it also had no action alone, and no alleles with visible effect have been reported. It might even be linked with the miniature locus, as is suggested by some data. But there are no general difficulties to an understanding of  $\text{mt}^{\text{e}}$  without unstable genes.

We turn now to  $\text{mt}^{\text{a}}$ . Here the other group of facts found in my case comes in. It turned out that within the  $\text{poi} : \text{dp}$  stock an allele of  $\text{bran}$  is present frequently, or occasionally, called  $\text{bran}^{\text{r}}$ , which, together with  $\text{svr}^{\text{poi}}$ , produces a wing with a phenotype like rudimentary and blistered. This combination is, as we saw, less viable and, if produced by mating  $\text{bran}^{\text{dp}}/\text{bran}^{\text{r}}$ , appears in less than one-fourth of the expected number, sometimes in only a few individuals. The allele  $\text{bran}^{\text{r}}$  alone produces a kind of truncated wing. The segregating  $\text{bran}^{\text{r}}/\text{bran}^{\text{r}}$ ,  $\text{svr}^{\text{poi}}$  flies breed true. Returning to our former comparison of the two cases, the  $\text{svr}^{\text{poi}}$  locus paralleled the  $\text{mt}$  locus in *virilis*;  $\text{bran}^{\text{dp}}$  paralleled an unknown locus in *virilis* with the discussed effect upon epistasis, otherwise producing a normal wing. If we had now, in addition, another allele of the latter (called  $e$ ) which, if homozygous, produces complete epistasis of its normal effect over the  $\text{mt}$  effect, thus paralleling the action of  $\text{bran}^{\text{r}}$ , true-breeding normal flies would result from this combination  $\text{mt}/\text{mt}$ ,  $e'/e'$ . A line containing  $e$  and  $e'$  with a chance for segregation of  $e/e'$  would be the complete explanation for Demerec's  $\text{mt}^{\text{a}}$ , throwing the "mutation" to normal.

*The alleles  $\text{bran}^{\text{a}}$  and  $\text{poi si}$ .*—In the section on mutation (below), mutational changes in a stock 369 of  $\text{px bl}$  will be related which parallel those reported in the Introduction. Among others, normal flies without  $\text{px}$  and  $\text{bs}$  appeared as before. Two such pairs were mated, the males showing indications of being  $\text{px}/+$ . The offspring consisted of (no. 4482):

+	50 ♀ 38 ♂
$\text{poi}$	12 ♀ 18 ♂
$\text{poi sing}$	11 ♀ 4 ♂
$\text{px}$	9 ♀ 11 ♂
$\text{px poi blist}$	15 ♀ 11 ♂

Thus both parents were heterozygous for  $\text{px}$ . The female was heterozygous for  $\text{poi}$  and the male had been  $\text{poi}$  without showing it. Moreover, something segregated which made the major part of the females and half of the males with  $\text{poi}$  singed (singed area on wings) or, in the presence of  $\text{px}$ , blistered. It turned out that, simultaneously with the "return mutation" of  $\text{px}$  and  $\text{bs}$ , a  $\text{poi}$  and a  $\text{bran}$  allele had arisen, the latter in a chromosome either with or without  $\text{px}$ . The pointed and singed individuals bred true and furnished the stock 4902  $\text{poi sing}$ , which bred true to pale, pointed flies, the majority of which had singed spots on the wings. Modifiers existed for the penetrance of singed which could be selected back when it became scarcer in the stock. Another stock containing the same constituents and  $\text{px}$  in addition was bred as 4918  $\text{poi sing px}$ ; here the singed wing area was frequently

replaced by a blister. Poi was clearly visible with px. The analysis made by crossing the stocks to +, different poi, and different bran alleles, and other tests, showed that a new bran and a new poi allele were present. The bran allele (like bran<sup>ap</sup>, above) alone has no visible effect in homozygous condition. It was called bran<sup>s</sup>. The poi allele alone cannot be distinguished from standard poi by its phenotype; it was called svr<sup>poi si</sup>. The combination of both homozygous (♂ hemizygous) gives the phenotype as described. If the compound bran/bran<sup>s</sup> is combined with poi si, the flies fluctuate from a pointed singed over a more or less soft blistered to an extremely blistered (when newly hatched) and completely folded wing. This and the other combinations show bran<sup>s</sup> to be a lower allele, but poi si higher than poi. The flies with poi si are also paler. If homozygous bran is combined with poi si, all flies are of the extremely blistered and folded type. In table 74 a number of such combinations are described. One remarkable feature of poi si is that it suppresses speck completely in the females but very incompletely in the males, whereas, other alleles have a tendency to the opposite behavior.

*The allele poi dish.*—From the same stock from which bran<sup>s</sup> and poi si had originated, another remarkable allele of poi was obtained, again in a strange combination. A pair of low px flies (the stock had previously been extreme px and bl) were mated. Three-fourths of the progeny (4489) were of the parents' own type, and one-fourth were pointed pale blistered and plexus flies exactly like those described as the combination bran<sup>s</sup> px/bran<sup>s</sup> px, poi si. The latter bred true (4679) in about half of their offspring, the other half not being blistered. F<sub>2</sub> was bred from both these types, and the offspring in two broods from each type were practically identical, namely, the px poi blist type, but with varying numbers of blisters (4948–51), showing that blistering had a fluctuating expression. In one of these (4948) from not blistered parents the majority of females had either an abnormal abdomen of the type found in higher bb alleles or in the a px deficiency (etched with various amounts of larval chitin present). One other brood contained some of these types. The brood 4938 bred true to type, including the abdomen effect (achi = achitinous). But in later generations the achi effect was somehow selected out and the established stock bred true to the new type without the persistence of larval chitin (stocks 5160 and 4949). The new character of this line is extremely pale color, far beyond that of poi, and a reduction of the dark bands at the posterior edge of the abdominal tergites. Simultaneously, the larger hairs of this fringe are reduced in number and size, and appear irregular, bent, or crooked; the small hairs of the tergites are also involved. In extreme cases all hair is straggling and disheveled, from which the symbol poi dish has been derived. It turned out that the abdominal achi effect had nothing to do with the disheveled hair effect, but was produced by a bb allele, which seems to have arisen simultaneously (see details below). This was proved by combining poi dish with bb. The abdominal effect is influenced by poi dish so far as the imaginal chitin tends to be localized at the posterior edge of the tergites, the same edge which poi dish alone affects. Poi dish contained also the bran<sup>s</sup> allele which, together with px and poi, produced the narrow blistered wings. Bran<sup>s</sup> also enhances the poi dish effect, which is most pronounced with homozygous bran<sup>s</sup>. Thus the new svr<sup>poi</sup> allele svr<sup>poi dish</sup>, appeared in the presence of bran<sup>s</sup> and together with a bb allele.

The same poi dish allele was obtained once more under similar circumstances. An achi stock had been built up from a combination of one bb allele introduced with a

TABLE 74

PHENOTYPES OF bran, poi, AND bran poi COMPOUNDS AND COMBINATIONS  
(Descriptive name in italics. If not mentioned, the first-chromosome locus includes both homozygous females and hemizygous males.)

2d chromosome	1st chromosome	Phenotype
bran/bran.....	+	<i>broad round</i> = bran
bran'/bran'.....	+	Normal, broader
bran <sup>2</sup> /bran <sup>2</sup> .....	+	<i>broad round</i> = bran (more angular?)
Bran/+.....	+	Like bran
bran'/bran.....	+	Like bran
bran'/arc.....	+	Almost like bran
Bran/bran.....	+	Like bran and Minute
Df apx/bran.....	+	exaggerated, ♂ frequently scalloped
Df apx/bran'.....	+	Like Df apx/+
Df apx/bran <sup>2</sup> .....	+	Like Df apx/+
+	svr <sup>poi</sup>	<i>pointed</i> (poi) (all svr alleles pale)
+	svr <sup>poi h</sup>	More pointed than svr <sup>poi</sup> (all svr alleles pale)
+	svr <sup>poi bl</sup>	pointed
+	svr <sup>poi s</sup>	Not very pointed, <i>soft</i>
+	svr <sup>poi sq</sup>	± pointed
bran/bran.....	svr <sup>poi</sup> or poi <sup>h</sup>	<i>soft blistered</i> and shortened
bran/bran.....	svr <sup>poi bl</sup>	<i>pointed blistered</i> = poi bl, long wings
bran/bran.....	svr <sup>poi s</sup>	soft blistered, many dumpy rudimentary blistered
bran/bran.....	svr <sup>poi sq</sup>	short dp-rud bl and also soft blist
Bran/bran.....	svr <sup>poi bl</sup>	soft blist and Minute
bran <sup>2</sup> /bran <sup>2</sup> .....	svr <sup>poi sq</sup>	broad incl. to square, truncated; most blistered = <i>bran blistered</i>
Df apx/bran <sup>2</sup> .....	svr <sup>poi sq</sup>	Minute, about one-half dumpy blistered, one-half like bran blist ±
bran <sup>2</sup> /bran <sup>2</sup> .....	svr <sup>poi</sup>	soft blistered
bran'/bran'.....	svr <sup>poi s</sup>	<i>slender</i>
bran/+.....	svr <sup>poi s</sup>	<i>slender and singed</i> (part of indiv.)
bran'/+.....	svr <sup>poi s</sup>	<i>slender and singed</i> (part of indiv.)
bran'/bran.....	svr <sup>poi s</sup>	<i>slender and singed</i> (part of indiv.)
bran <sup>2</sup> /+.....	svr <sup>poi s</sup>	Some slender with basal blisters = <i>slender blistered</i>
bran <sup>2</sup> /bran <sup>2</sup> .....	svr <sup>poi s</sup>	slender blistered-soft blist
Bran/bran <sup>2</sup> .....	svr <sup>poi s</sup>	<i>rudim. blist</i>
bran <sup>dp</sup> /bran <sup>dp</sup> .....	+	Normal
bran <sup>dp</sup> /+.....	+	Normal
bran <sup>dp</sup> /bran.....	+	Normal
bran <sup>dp</sup> /bran <sup>r</sup> .....	+	broad round toward dumpy
bran <sup>r</sup> /bran <sup>r</sup> .....	+	broad round toward dumpy
bran <sup>r</sup> /+.....	+	Normal
bran <sup>r</sup> /bran.....	+	broad round
bran <sup>dp</sup> /bran <sup>dp</sup> .....	svr <sup>poi</sup>	<i>pointed dumpy</i> = poi:dp, transitions poi-poi:dp -dp-dp blist
bran <sup>dp</sup> /+.....	svr <sup>poi</sup>	pointed
bran <sup>dp</sup> /bran.....	svr <sup>poi</sup>	pointed
bran <sup>dp</sup> /bran <sup>r</sup> .....	svr <sup>poi</sup>	pointed dumpy, etc. (poi:dp)
bran <sup>r</sup> /bran <sup>r</sup> .....	svr <sup>poi</sup>	rudimentary blistered and soft blistered (more former)

TABLE 74—(Continued)

2d chromosome	1st chromosome	Phenotype
bran <sup>r</sup> /+ . . . . .	svr <sup>poi</sup> . . . . .	pointed
bran <sup>r</sup> /bran . . . . .	svr <sup>poi</sup> . . . . .	rudimentary and soft blistered (more latter)
bran <sup>tr</sup> /bran <sup>s</sup> . . . . .	♀ poi/poi <sup>sq</sup> ♂ poi . . . . .	♀ like poi:dp but majority poi; ♂ the same, still fewer not poi
bran, bran <sup>s</sup> . . . . .	♀ poi/po <sup>sq</sup> ♂ poi . . . . .	♀ dumpy and dumpy blist; ♂ few the same, most soft blist
bran <sup>dp</sup> /bran' . . . . .	♀ poi'poi <sup>s</sup> . . . . .	<i>slender</i>
bran <sup>dp</sup> /bran <sup>s</sup> . . . . .	♀ poi <sup>s</sup> /poi ♂ poi <sup>s</sup> . . . . .	Like poi:dp, ♀ poi:dp singed
bran'/bran <sup>s</sup> . . . . .	♀ poi <sup>s</sup> /poi <sup>sq</sup> ♂ poi <sup>s</sup> . . . . .	♀ between bran blist and soft blist; ♂ most soft blist
bran'/bran <sup>s</sup> . . . . .	♀ poi/poi <sup>sq</sup> ♂ poi . . . . .	soft blist
bran <sup>r</sup> /bran <sup>s</sup> . . . . .	♀ poi/poi <sup>sq</sup> ♂ poi . . . . .	♀ dumpy-bran blist; ♂ dumpy blist-soft blist
bran <sup>dp</sup> /bran <sup>dp</sup> . . . . .	♂ poi <sup>sq</sup> . . . . .	dumpy blist (?)
bran <sup>r</sup> /bran <sup>r</sup> . . . . .	♂ poi <sup>sq</sup> . . . . .	soft blist (?)
bran <sup>s</sup> /bran <sup>s</sup> . . . . .	♀ poi <sup>s</sup> /poi <sup>sq</sup> . . . . .	Between soft blist and bran blist
bran <sup>db</sup> /bran <sup>s</sup> . . . . .	♀ poi <sup>s</sup> /poi <sup>s</sup> . . . . .	soft blistered
bran <sup>db</sup> /bran <sup>s</sup> . . . . .	♀ poi <sup>s</sup> /poi <sup>sq</sup> . . . . .	dumpy blistered
bran <sup>db</sup> /bran <sup>db</sup> . . . . .	poi <sup>sq</sup> . . . . .	dumpy blistered (sometimes var. to soft bl.)
bran <sup>db</sup> /bran . . . . .	♀ poi <sup>s</sup> /poi . . . . .	soft blistered
bran <sup>db</sup> /bran . . . . .	♀ poi <sup>sq</sup> /poi . . . . .	dumpy blistered
bran <sup>db</sup> /bran . . . . .	♀ poi <sup>sq</sup> /poi <sup>sq</sup> ♂ poi <sup>sq</sup> . . . . .	soft folded (blist)
bran <sup>db</sup> /bran <sup>db</sup> . . . . .	poi/+ or +/+ . . . . .	bran
bran <sup>db</sup> /bran . . . . .	poi <sup>sq</sup> /+ . . . . .	bran
bran <sup>s</sup> /bran <sup>s</sup> . . . . .	+ . . . . .	Normal
bran/bran <sup>s</sup> . . . . .	+ . . . . .	Normal
bran <sup>s</sup> /bran <sup>s</sup> . . . . .	+ . . . . .	Normal
bran/bran <sup>s</sup> . . . . .	♀ poi/poi ♂ poi . . . . .	pointed, some singed or blistered
bran/bran <sup>s</sup> . . . . .	poi si . . . . .	poi sing-soft blist-very blist folded
bran <sup>s</sup> /bran <sup>s</sup> . . . . .	poi si . . . . .	<i>pointed singed</i>
bran/bran . . . . .	poi si . . . . .	Very blist folded
bran <sup>s</sup> /bran <sup>s</sup> . . . . .	poi si . . . . .	pointed soft, blister in V
bran <sup>r</sup> /bran <sup>s</sup> . . . . .	poi si . . . . .	± pointed singed
bran <sup>r</sup> /bran <sup>s</sup> . . . . .	poi si . . . . .	poi sing-soft blist-very blist folded
a px/a px . . . . .	poi si . . . . .	pale plexus blistered arc
bran <sup>s</sup> px/bran <sup>s</sup> px . . . . .	poi si . . . . .	pale pointed plexus blistered
+/+ . . . . .	poi si . . . . .	pale pointed
bran <sup>s</sup> /+ . . . . .	poi si . . . . .	pale pointed
+/+ . . . . .	poi dish . . . . .	<i>very pale, pointed disheveled</i> ♀ more than ♂
bran <sup>s</sup> px/bran <sup>s</sup> px . . . . .	poi dish . . . . .	narrow, plexus, blistered, pointed, very pale, very disheveled
bran/bran . . . . .	poi dish . . . . .	soft blistered, dwarfish, very pale, etc.
bran <sup>s</sup> px/bran <sup>s</sup> px . . . . .	poi si/poi dish . . . . .	Like poi dish with bran <sup>s</sup>
bran <sup>s</sup> /bran <sup>s</sup> px . . . . .	♀ poi/poi dish ♂ poi dish . . . . .	Only few ♀ singed, poi dish ± dominant, ♂ var. to soft blist
bran/bran <sup>s</sup> px . . . . .	poi/poi dish . . . . .	poi dish ± dom; few ♀ blist in V
bran <sup>r</sup> /bran <sup>s</sup> px . . . . .	poi <sup>sq</sup> /poi dish . . . . .	poi dish ± dom; no blisters
bran <sup>s</sup> /+ . . . . .	poi/poi dish . . . . .	poi dish ± dom
bran <sup>s</sup> /bran <sup>s</sup> . . . . .	poi dish/poi dish . . . . .	poi dish type but dumpy with basal blister like bran blist-poi bl-soft blist
bran <sup>r</sup> /bran <sup>r</sup> . . . . .	+ . . . . .	Like bran, varying toward ±
bran <sup>r</sup> /bran <sup>s</sup> . . . . .	+ . . . . .	Between + and bran
bran <sup>r</sup> /bran <sup>r</sup> or <sup>s</sup> . . . . .	poi bl . . . . .	pointed ± singed-blist
bran <sup>s</sup> /bran . . . . .	poi dish . . . . .	poi dumpoid-poi sing-almost soft blist
bran <sup>s</sup> /bran' or <sup>dp</sup> . . . . .	poi dish . . . . .	Very few sing
bran/bran . . . . .	poi dish . . . . .	soft folded, blist ?

pointed X chromosome and another introduced by a *px sp* (see section on *bb*), a stock in which all females had the *achi* abdomen together with short bristles. This stock was outcrossed to standard pointed, and in the offspring *poi* dish appeared, i.e., again in the presence of the *arc*, *bobbed*, and the *svr<sup>poi</sup>* mutant loci. Analysis showed it to be identical with the 5160 *poi* dish allele. More details will be given in the section on mutation. The new mutant *poi* dish was checked for allelism to *poi* and for *sp* suppression, with positive results. The presence of *bran*<sup>3</sup> was revealed by the same checks as are presented in the foregoing section. The irregular presence of a *bb* allele with no visible effect when alone and only a slight compound effect with standard *bb* was also found. As these crosses (actually very elaborate because originally the relations of *bran*<sup>3</sup> to *achi* and *bb* were not known) do not show anything new; they will not be tabulated; and only some of the combinations are entered in the table (74) of phenotypes.

When these experiments were performed, still another *bran* allele was found, *bran*<sup>4</sup>. As not much is known of it, only the known phenotypes have been entered in table 74. Its origin will be discussed below in the section on mutation.

Table 74 tabulates all the phenotypes of the *bran* or *poi* alleles and their combination effects studied in the foregoing pages.

*Bobbed in the pointed stocks*.—We have already mentioned the fact that a *bobbed* allele was found in the pointed stocks, but no short bristles, the bristles actually tending to be longer than usual. But in the offspring from pair matings, flies were sometimes found with etched abdomen, which is one of the phenotypical expressions of the *bb* mutants, though usually this character is found only in the higher alleles with shortened bristles. Here it was present with normal bristles. As this etching is the lowest expression of incomplete imaginal chitinization, i.e., persistence of larval chitin, we call the phenotypes beginning with a little etching of the tergites and leading through all transitions to abdomina with nothing but larval chitin the *achi* type (achitinous). To give an example for *svr<sup>poi</sup>*: Among the offspring of 20 pair matings, 8 contained these etched (low *achi*) flies (only females), namely, once 1 among 43, once 15 among 71, once 5 among 72, and 5 times, the majority of the females. By crossing to the *bb* alleles, it was found that an allele of *bb* was present which in homozygous condition had no bristle effect, though some *achi* effect, as stated; but in compounds with other *bb* alleles the bristle effect became visible.

When it was found that *px bl*, as well as *svr<sup>poi</sup>* of completely unrelated origin, sometimes also contained a *bb* allele, and furthermore, that remarkable relations obtained between the *svr* alleles and *bb* (see the foregoing section on *poi* dish), we made a closer study of the *bb* locus in the stocks of *svr* alleles. This study was partly facilitated, partly complicated, when it turned out that the standard a *px sp* stock contained *bb* alleles which in compound with those from *poi* produced a high and even extreme *achi* and *bb* phenotype, though otherwise they had hardly any visible effect. As tester stocks two *bb* stocks were used, kindly furnished by Professor Curt Stern, one marked with yellow garnet (*y g bb*), with a very small phenotypic effect, the other a *bb*<sup>1</sup> stock carried as *CIB/w<sup>+</sup>bb<sup>1</sup>*, with rather variable effect but powerful in compounds (probably a deficiency). To avoid lengthy descriptions, all phenotypes of homozygous or compound females relevant for the present analysis are presented in table 75. A comparison of the different phenotypes explains the results of the genetical analysis. It ought to be added that the phenotype is rather modifiable both

TABLE 75  
PHENOTYPES OF bb ALLELES AND COMPOUNDS

No.	bb allele		Origin		Phenotype ♀	
	X <sup>1</sup>	X <sup>2</sup>	X <sup>1</sup>	X <sup>2</sup>	Bristles	Abdomen
1	bb.	bb.	y g bb.	y g bb.	+ - bb 1.	+ - achi 1
2	bb <sup>1</sup> .	bb <sup>1</sup> .	CIB/bb <sup>1</sup> .	bb <sup>1</sup> .	+ and some bb 3.	+ - achi 1
3	bb.	bb.	y g bb.	y g bb.	bb 2-3.	achi 3-4
4	bb.	bb.	y g bb.	poi dish 5145.	bb 1-2.	+ - achi 2
5	bb.	bb <sup>poi</sup> .	y g bb.	poi dish 5145.	bb 4-5.	achi 5
6	bb <sup>poi</sup> .	bb <sup>poi</sup> .	CIB/bb <sup>1</sup> .	poi dish 5145.	bb 2-3.	achi 2
7	bb <sup>poi</sup> .	bb <sup>poi</sup> .	CIB/bb <sup>1</sup> .	poi dish 5145.	bb 5.	achi 4-5
8	bb.	bb <sup>px sp</sup> .	y g bb.	5144 a px sp achi.	bb 1-2.	+ - achi 2
9	bb <sup>1</sup> .	bb <sup>px sp</sup> .	CIB/bb <sup>1</sup> .	5144 a px sp achi.	bb 4.	achi 4
10	bb.	bb <sup>px sp</sup> .	y g bb.	a px sp.	bb 2.	achi 1-3
11	bb <sup>poi</sup> .	bb <sup>px sp</sup> .	CIB/bb <sup>1</sup> .	a px sp.	bb 2-4.	achi 3-4
12	bb <sup>poi</sup> .	bb <sup>px sp</sup> .	poi dish 5145.	a px sp.	bb 3-4.	achi 3-4
13	bb <sup>poi</sup> .	bb.	From achi ♂ 6317.	y g bb.	bb 2-4.	achi 5
14	bb <sup>poi</sup> .	bb <sup>poi</sup> .	5144 a px sp achi.	poi sq 5121.	(2d chr. a/bran <sup>2</sup> ) bb 5.	achi 2
15	bb <sup>poi</sup> .	bb <sup>poi</sup> .	5144 a px sp achi.	poi sq 5121.	(2d chr. a/bran <sup>2</sup> ) bb 2-3.	achi 1-2
16	bb.	bb <sup>poi</sup> .	y g bb.	poi sq 5121.	(2d chr. a/bran <sup>2</sup> ) bb 1-2.	achi 5
17	bb <sup>poi</sup> .	bb <sup>poi</sup> .	CIB/bb <sup>1</sup> .	poi sq 5121.	(2d chr. a/bran <sup>2</sup> ) bb 5.	like bb
18	bb <sup>poi</sup> .	bb <sup>poi</sup> .	From crossover y g bb a	and poi	like bb.	+
19	bb <sup>1</sup> .	bb.	CIB/bb <sup>1</sup> .	a px sp.	(2d chr. a/a) +	achi 4-5
20	bb <sup>poi</sup> .	bb <sup>poi</sup> .	poi dish 5145.	poi dish 5145.	(2d chr. bran <sup>2</sup> /bran <sup>2</sup> ) bb 4-5.	achi 1-2
21	bb <sup>poi</sup> .	bb <sup>px sp</sup> .	poi dish 5145.	poi dish 5145.	(2d chr. bran <sup>2</sup> /bran <sup>2</sup> ) bb 1.	achi 1-2
22	bb <sup>poi</sup> .	bb <sup>px sp</sup> .	poi dish 5145.	poi dish 5145.	(2d chr. bran <sup>2</sup> /bran <sup>2</sup> ) bb 2.	achi 1-2
23	bb <sup>px sp</sup> .	bb <sup>px sp</sup> .	5144 a px sp achi.	5144 a px sp achi.	(2d chr. bran <sup>2</sup> ) + and bb 1.	+ - achi 1
24	bb <sup>1</sup> .	bb <sup>px sp</sup> .	CIB/bb <sup>1</sup> .	5144 a px sp achi.	(2d chr. a/a) bb 2-4.	achi 3-4
25	bb.	bb.	y g bb.	y g bb.	(2d chr. all bran alleles) +	+
26	bb <sup>1</sup> .	+	CIB/bb <sup>1</sup> .	Diff. stocks.	(2d chr. all bran alleles) like bb/+	like bb/+ +/+
27	bb <sup>poi</sup> .	bb <sup>poi</sup> .	Diff. stocks.	Diff. stocks.	bb 4-5.	achi 3-5
28	bb <sup>px sp</sup> .	bb <sup>px sp</sup> .	5144 achi a px sp.	5144 achi a px sp.	bb 4-5.	achi 3-5
29	bb <sup>poi</sup> .	bb <sup>poi</sup> .	Diff. stocks.	Diff. stocks.	+	+ - achi 1
30	bb <sup>px sp</sup> .	bb <sup>px sp</sup> .	5144 achi a px sp.	5144 achi a px sp.	+ - bb 1	+ - achi 1
31	bb <sup>poi</sup> .	bb <sup>poi</sup> .	Diff. stocks.	Diff. stocks.	Lethal.	Lethal
32	bb <sup>px sp</sup> .	bb <sup>px sp</sup> .	5144 achi a px sp.	5144 achi a px sp.	Lethal.	Lethal

## Legend:

bb 1-5: little, 1/4, 1/2, 3/4, complete, shortening of bristles.

achi 1-5: little etching, more etching, large patches of larval chitin, more larval than imaginal chitin, almost no imaginal chitin.

5144 a px sp achi = stock with high incidence of bb achi derived from backcrosses (a px sp × poi) × a px sp containing compounds of the respective bb alleles.

5145 poi dish = stock of same derivation containing the later-arisen mutant poi dish and freed from a px sp by outcrossing to poi; contains both bb alleles from poi.

by environment and by genetic modifiers, and hence that many repetitions of crosses and selections in extracted stock were required for determining the genetical basis of the observed results.\*

In the a px sp stock the bristles are normal, but individuals with more or less etched abdominal tergites, i.e., beginning and low achi, are frequent. Occasionally, a fly appears with higher-grade achi, and once a female extreme achi and bobbed was found. The explanation will be given below. As table 75 (nos. 8-13) shows, most flies from a px sp stock contain a bb allele, some are heterozygous for it, and some are free from it. This allele in homozygous condition has no bristle effect, which is also true for other alleles; but the bobbed character appears in all compounds with other alleles, also in those which themselves have no bristle effect. This strange behavior was found for all lower bb alleles used in this work, and only the higher ones showed a homozygous effect. The achi effect of the compounds generally parallels the bristle effect, though one might say that as a rule the shortening of the bristles is one step ahead of the increase in the achi character. But it turned out that the presence of homozygous arc acts as an enhancer of the achi effect. This is remarkable because a deficiency in the arc region—Df(2)a px—also has an achi effect, together with the bristle effect of the Minutes. We shall return to this point later. It further turned out that sometimes a px sp contains a higher bb allele which is either completely or almost lethal in homozygous condition, and which in compounds reduces viability considerably. The compound of the two alleles looks high to extreme bb and achi, especially in the presence of arc. There is reason to believe that this allele originates frequently by mutation in the stock. Males with the high allele are viable, and thus apparently true-breeding lines may be obtained from compound females and genetically high males, the homozygous high females being lethal. We call these alleles  $bb^{a\ px\ sp}$  and  $bb^{a\ px\ sp\ h}$ , the former being a little higher than the standard bb used here (y g bb). Both give the expected exaggerated effect in compound with  $bb^1$ . Also, males with the high allele  $bb^{a\ px\ sp\ h}$  have a tendency to show beginning achi in the genital segment (up to 10 per cent of the males).

The bb allele present in poi turned out to be a little lower than  $bb^{a\ px\ sp}$ . Again, it has no bristle effect in homozygous condition, but in compound with  $bb^{a\ px\ sp}$ , i.e.,  $bb^{a\ px\ sp}/bb^{poi}$ , a low bristle and achi effect appears, and a high one in compound with  $bb^{a\ px\ sp\ h}$ . Whenever, from a cross involving these alleles, homozygous  $bb^{a\ px\ sp}$  or  $bb^{poi}$  segregates together with the compound, we have one-half normal, one-half bb achi flies, a fact which caused much confusion before the compound action had become clear.

Both in crosses between poi and a px sp and in selections of poi exhibiting the achi character, a high allele of bb was sometimes also found in the poi chromosome. Thus, it was found when poi dish originated (see above), and it was also present occasionally with other poi alleles, especially poi sq. Its action upon the phenotype is about midway between bb and  $bb^{a\ px\ sp\ h}$ . Thus the increasing order of the alleles derived from the bb and achi effect is bb (y g bb)— $bb^{poi}$ — $bb^{a\ px\ sp}$ — $bb^{poi\ high}$ — $bb^{a\ px\ sp\ h}$ .

As we have said, arc enhances the achi effect. Therefore, all bran alleles were combine with bb and  $bb^1$  to compare their effect. It turned out that no enhancing was observable except with bran<sup>2</sup> (which is a one-band deficiency) in compound with arc. But, strangely enough, homozygous bran<sup>2</sup> did not enhance the effect. We

\* Some of the facts presented here have been made the subject of a special contribution to Amer. Nat., December, 1944.

therefore also tested the *Df(2)a px* (which alone is Minute with a little achi) with all available *bb* alleles, but we could not discover any enhancing effect in the presence of homozygous or heterozygous bobbed alleles.

Finally, the phenotypic combination of *bb<sup>poi h<sup>1st</sup></sup>* in the presence of *poi dish* must be mentioned. Whereas the high-grade achi abdomina show all kinds of irregular patches of imaginal chitin on the basis of white sections of larval chitin, in the presence of *poi dish* the imaginal chitin is always preserved dorsally at the posterior edge of the tergites. We have described above the effect of *poi dish* upon the chitin and bristles of these same edges, an action which thus in some way controls also the localization of the achi effect.

At the time of the discovery of *bb* it was found (see Morgan and Bridges, 1916) that hereditary bobbed males occasionally appeared, which was interpreted as a mutation of the *+<sup>bb</sup>* allele in the Y chromosome to *bb*. The same phenomenon was observed repeatedly both in *bb<sup>++</sup>* and in *bb<sup>poi</sup>*, and also in the higher alleles. The responsibility of the Y chromosome was easily ascertained in crossing to *y* and further generations in which the Y chromosome alternates from males to females with the consequent effect upon males. But it seems that the explanation by mutation in the X chromosome does not suffice, as cases were found where the *bb* achi males contained an X chromosome without *bb*. Other peculiarities, such as a huge enhancing effect upon *bb* males by a *Dichaete* third chromosome, are only mentioned. Still more remarkable is the fact that males with *bb<sup>1</sup>* in the X chromosome and the mutated Y which makes *bb* visible are normal. But such facts do not belong here.

Finally, it happens very frequently that in crosses which ought to yield only normal or low *bb* flies a few individuals with high *bb* achi appear. When tested, they always turn out to be compounds of the low *bb* introduced into the cross and a high *bb* allele which must have arisen by mutation, which thus seems to be a rather frequent occurrence in *bb* chromosomes whether derived from our *poi* stocks or not.

I point out again that *bb* was found in *px bl* and in *poi*, *poi h*, *poi sq*, all of more or less different origin, and that a relation was repeatedly found between the appearance or presence of *bb* and the mutation at the *bran* and *poi* loci. The data are presented in the appropriate sections.

#### d. NOTES ON THE PHENOTYPE OF WING SHAPE

In the foregoing pages we have described a number of wing types which are obtained when mutant loci for pointed and for broad and shortened wings collaborate. Different conditions obtained according to the alleles and loci involved—conditions which might be described in general terms as different degrees of epistasis including threshold phenomena. Similar phenotypes are produced also by the collaboration of different loci with visible effects comparable to those of *poi* and *bran*. In all cases the mutant predominantly affects the posterior wing margin. One group of mutants, namely, the pointed alleles at the silver locus (first chromosome), and the lanceolate alleles (second chromosome), produce more or less pointed wing tips in the lower grades and a retraction around the end of the fifth longitudinal veins, which results in narrow slender wings in the higher grades. I have shown in an earlier work (Goldschmidt, 1937) that such wings are normal at the time of pupation but soon afterward produce the pointed tip by a kind of contraction, which I considered to be associated with the histolysis of subepidermal tissue, whereas Waddington



(1942) thinks that only contraction is involved. Furthermore, the pointed phenotype can actually be obtained as a so-called phenocopy by treating normal late larval and early pupal stages with heat shocks (Goldschmidt, 1935). The phenocopies obtained in a complete series of grades are exact copies of the phenotype of the first-mentioned mutants. The other group of mutants studied here, namely, the *bran* alleles in the *arc* region of the second chromosome and rudimentary in the first chromosome, produce more or less truncated wings (like the mutant *dumpy* and other similar ones), wings in which the tip shortens, and the posterior edge curves in giving the wing a broadened appearance. Moreover, the wing appears to grow only to more or less of its full length, thus producing all transitions from a long, slightly truncated, to a very short rudimentary wing of essentially the same form. It has been shown by Auerbach and by me (Auerbach, 1936; Goldschmidt, 1935) that truncated wings are normal at the time of pupation, but afterward retract in their sheath to form a truncated and shortened wing, while a cytolysis of subepithelial tissue takes place. Waddington (1940), however, assumes that the wing appears normal at the time of pupation only because it is overinflated, and that it later retracts to a predetermined *dumpy* form. This is not the place to discuss these different interpretations of identical facts, but I have many reasons to doubt Waddington's interpretation. Also, this type of truncated wing may be produced as phenocopy by heat shocks (Goldschmidt, 1929, 1935; confirmed since by many authors). In this case all transitions may be obtained from a pointed-like wing to one intermediate between pointed and *dumpy* to typical truncation, thus showing that the sharpening and truncating features are based more or less on closely related embryological processes (with which my embryological observations, *loc. cit.*, agree).

This comes out clearly when wing-broadening (*bran* and alleles) and wing-sharpening mutants (pointed and alleles, *lanceolate*) are present simultaneously; in this case the sharpening process in development combining with the broadening one may lead to a regular truncation. In detail, however, a great many different possibilities are realized, depending upon the amount of epistasis of the two loci involved, i.e., their relative influence at the critical time in development, which probably means definite threshold conditions (see discussion of the *poi:dp* phenotype above). At the one end of the line of possibilities actually realized we find the combination of *svr<sup>poi</sup>* with rudimentary. As reported above, both pointed and a rudimentary allele *r<sup>px bl</sup>* arose simultaneously from *px bl* stock. The *r<sup>px bl</sup>* was at once mated to *y* and kept as a stock. One such stock (see below) always showed short rudimentary wings, whereas others had long, *dumpy*-like wings, and one actually reverted to a normal phenotype though genetically it was still *r<sup>px bl</sup>*. The short rudimentary turned out to contain also pointed, which bred with *y*, could not be lost by crossing over. In this case either rudimentary, i.e., extreme truncation, was completely epistatic over pointed, or, more probably, truncated was epistatic, but pointed acted simultaneously in the combination by still further shortening the rudimentary wing besides producing blisters. We remember that exactly the same phenotype, called *rud blist*, was also the result of the combination of certain *bran* alleles and compounds with *svr<sup>poi</sup>* and alleles (see table 74). In these combinations of two different loci, one for sharpening, one for truncating, the wings were symmetrical at the high level of shortening described as rudimentary. At the same end of the epistatic series are such combinations as *bran blistered* (see table 74) with symmetrical broad

wings (sometimes just beginning truncation), which may or may not show the influence of the poi allele by a symmetrical or one-sided blister. At the other end of the epistatic series, i.e., epistasis of the poi effect (sharpening) over the truncation effect, are such combinations as poi blist where the allele  $svr^{poi\ bl}$  with bran produces pointed wings, partly with blisters but without any truncation, and others found in table 74. Between these two extremes all grades of intermediate and variable epistasis relations can be found, and among them asymmetry is a typical feature. Thus bran/bran,  $svr^{poi}$  is the type soft blistered. Most wings are pointed but at the same time shortened (and blistered). All transitions appear from pointed to broader wing tips. A small, varying percentage has either one wing short pointed blistered, the other truncated or even rudimentary, or both wings truncated or rudimentary. In the latter case there is usually no clear epistasis, but a kind of compromise between the two wing types. The embryological processes must be such that near a certain threshold the truncating influence wins out, and this threshold is obviously so narrow that it may be passed by one wing and not the other, owing to the small right-left differences in differentiation, well known to the embryologist. For an interpretation of all these phenomena in terms of the physiology of development see the discussion in my book *Physiological Genetics* (1939), where a dynamic explanation is derived from other cases of the same general type.

A last group shows the developmental reactions and processes of sharpening and truncation, both limited to so short a time of alternative determination (which means the same as a narrow threshold for epistasis) that a variation is found in epistasis from individual to individual, from pointed through all transitions to truncated wings, with, in the transitory group, asymmetrical behavior of the two wings. This group is represented by the combination of bran<sup>49</sup> with pointed (the poi:dp case), but it is also represented by a combination of bran with a completely different locus for pointed wings, lanceolate (ll) in the same second chromosome (see p. 309). Finally, also, the combination of rudimentary with pointed, which is usually of the phenotype short rudimentary blistered, may show, in certain genetic conditions to be reported below, all transitions, symmetrical and not, from a pointed to a rudimentary wing. Thus, this entire group of phenotypes presents a good illustration of the phenomenon of epistasis based upon an embryological threshold at the time of wing differentiation. It shows the different ability of the individual alleles (or, more correctly, their effects) to pass the threshold, and of others to control the more or less narrow time limit in which determinative processes are decided in development, a situation which may produce intermediates and mosaics. We shall not go into further detail here. A comparison of the facts with others analyzed in the book just mentioned shows that a beautiful case could be made out for our interpretation of all such phenomena in terms of interlaced reaction velocities.

#### e. THE SALIVARY CHROMOSOMES OF THE poi AND bran ALLELES, IN THE svr AND arc REGION

On the basis of the deficiency tests the silver locus is expected to be located to the left of 1C in the first chromosome.<sup>5</sup> In all alleles, including those produced by X rays,

<sup>5</sup>Demerec, in a list of loci located in the salivaries (*Carnegie Inst. Yearbook* 41), mentions svr as located in 1B5, 6, which is rather far to the left of the Eld break.

the region left of Bld is perfectly normal. But in some alleles the region farther to the right, between the Bld break and the bulb, shows typical disturbances. The only one of these which can be described with certainty is found in the allele  $svr^{poi}$ . Here a 2- (4-) band inversion of 1E1-4 is always found. These bands are described in Bridges' standard map (1938) as two double bands, the one to the left being thinner, that to the right being rather thick. I think that there exist actually only two bands, the double nature of which is not always visible, being based upon a partial separation of the two groups of perultimate chromomeres of which the bands are constituted (see Kodani, 1942; Goldschmidt and Kodani, 1943). In the heterozygote this inversion looks as pictured in plate 30, figures 5 and 6. One cannot always be sure that the interpretation as a minute inversion is correct. But in the homozygote the order of the thin and thick bands 1-2, 3-4 is clearly reversed, as shown in plate 30, figure 7. Therefore we feel confident that the interpretation is correct.

In some of the other alleles, namely,  $svr^{poi}$ ,  $svr^{poi}^{sq}$ ,  $svr^{poi}^{dih}$ , an abnormality of the same bands is found in practically all X chromosomes (this is never true of the controls), a structure which offers great difficulties of interpretation. The order of the bands 1-2, 3-4 is normal. But the thick band 3-4 is distorted in a way that is difficult to describe. When focusing high and low the band seems normal, but in between a kind of cross or X figure appears, an apparent crossing of 1, 2 with 3, 4. This seems to be the result of two features: first, the arrangement of the chromatin on the surface of the disk-like band (like a ring), and secondly, a dislocation of the two halves of this ring belonging to the two homologous chromosomes by a twist in opposite directions. This means that, viewed from above, the upper rim of the disk in one chromosome is shifted to the right and that of the other chromosome to the left, so that a kind of figure 8 becomes visible. This is pictured in plate 29, figures 4-7, where the same chromosome is drawn at different foci. Our interpretation of these typical aspects, which can hardly be drawn as they appear in focusing, is that the distortion within the disk represents a one-band inversion. This requires the assumption that the left and right surfaces of a single disk are serially different. The new discoveries on the structure of the individual disk (see Kodani, and Goldschmidt and Kodani) make such an interpretation possible, since they revealed the presence of a coil of genonema with an attached series of perultimate chromomeres in a disk (each one double). An inversion of one disk would change the direction of the coil, creating a tension in the state of synapsis. (Consult fig. 6, in Kodani's paper.) Homozygous chromosomes were always normal.

In  $svr^{poi}^{h}$  a different structure is found, again for the same bands. We did not succeed in finding a satisfactory interpretation. The only certain fact is that these bands are not normal. A few of the varying aspects are figured in plate 30, figures 1-4. Sometimes the structure appears to be in the twisted condition just described. The bands seem to be imbedded in a darker-staining substance.<sup>7</sup>

It is remarkable that in some of the  $poi$  alleles a clear inversion or less clear abnormalities are found about 6 bands beyond the supposed location of silver. On the basis of our knowledge of the adjacent yellow and scute regions (see discussion in Goldschmidt, 1944) we may assume that the entire region between the scute

<sup>7</sup> A special discussion of these features is given in R. Goldschmidt and A. Hannah, Proc. Nat. Acad. Washington, 1944.

segment and the bulb is involved in producing the poi phenotype (svr itself has completely normal bands) in the sense discussed in the paper mentioned.

The bran alleles involve the arc region, which is known to be located in the second chromosome between 58B and 58E (see fig. 1 in Bridges, 1937). Actually, the allele bran<sup>3</sup> contained in the combination with poi square described as broad blistered is a clear one-band deficiency for what is called by Bridges 58D6, 7, but which appeared in our slides as only a single band. Figures 1-3, plate 29, represent this deficiency, which was always found in good slides. None of the other bran alleles showed any abnormalities, but unfortunately all the dominant Bran could not be checked.

f. THE CHROMOSOME SECTIONS CONTAINING THE poi AND bran ALLELES  
AND THE ALLELISM WITH SVT AND A (arc)

*The silver region.*—The section of the X chromosome in which the silver alleles are located contains a number of loci the interrelations of which are not completely clear. In addition to silver there is a group of so-called suppressors, namely, su-s, a suppressor for sable; su<sup>2</sup>-s a suppressor for both sable and vermillion; su<sup>3</sup>-s, a suppressor for speck also. All three are described as allelic to one another. Another one, su<sup>88-v pr</sup>, suppresses vermillion and purple, but has not been tested for allelism with the others. There is, further, in this region the break of the Blond translocation. As the silver alleles are pale, all these loci have in common an effect upon pigmentation which is suppressed or diluted either in the cuticle of the entire surface of the fly, excluding hair (silver and alleles), or only in hair and bristles (Blond), or only when another recessive mutant is present which produces a specific pigmentation, namely, sable, vermillion, speck. Sometimes, too, a trident is present (svr); this may be the result of transparency of the cuticle overlying the pigmented epidermal pattern (which itself is probably based upon the arrangement of muscle insertions). Finally, the silver group has the additional action of producing pointed and sometimes soft-textured wings, in different degrees, if at all. Nothing is known of crossing over between all these loci, and it would be difficult to study it except for the specific suppressors. Actually, the phenotypical effects completely overlap (except Blond), since silver and alleles are always pale but not always pointed, silver has or has not the trident which is absent in its alleles, svr does not act as a suppressor, and the suppressors are neither pale nor pointed; but all silver alleles of the poi series are suppressors for speck, incomplete suppressors for sable, not for vermillion. (One, poi si, suppresses sp only in females.) This situation reminds one very much of the condition in the yellow and scute sector near by, e.g., different shades of yellow, yellow or dark bristles, etc., with different alleles and rearrangements. For the yellow and scute cases it is known (see discussion in Goldschmidt, 1944) that sections of about 6-8 bands exist within which mutants without visible chromosome disturbance and those based upon position (rearrangement) effects form a series of alleles. Thus, one should expect a similar situation in the svr region which immediately follows the yellow and scute sections to their right.

The break of the Bld translocation is located in the salivary chromosomes between bands 1C3 and 4. According to Bridges, silver as well as the suppressors are located to the left of the break. For the study of this region there are available, also, L. V. Morgan's deficiency of the tip of chromosome 1, including silver; Dobzhansky's

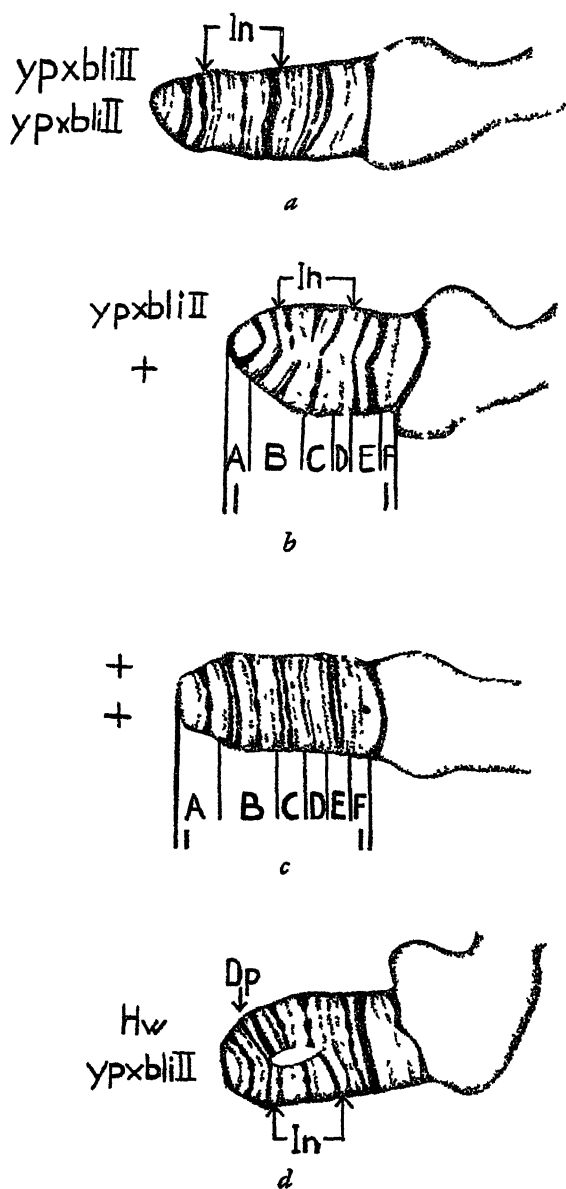


Fig. 3.  $I(1)y^{xbl}$  = Inversion yellow-px bl in first chromosome 1. *a.* Homozygous inversion. *b.* Heterozygous inversion. *c.* Normal chromosome for comparison. *d.* Inversion opposite Hw duplication. M. Kodani del.

duplication of this region (101), which includes svr and the suppressors; and a new small inversion with yellow phenotype, with the left break in the yellow region, the right break identical with the Blond break (see p. 388, text fig. 3). The pointed alleles which show changes in the salivary chromosomes have the breaks to the right of the Bld break (see above, pp. 395 ff.).

We studied different combinations and compounds to find out the relations between these different mutants. Table 76 contains some of the information. (Only the  $su^2-s$  was tested among the suppressors.) The table shows that the phenotypic allelism is more or less clear. It is clear for the silver alleles, but no visible allelism with the  $su-s$  exists (the phenotype of this being normal), though the suppressor action for sable is common to both and to the compound, i.e., it is allelic. The haplo effects of the deficiency  $y-svr$  are clear, but exaggeration is absent, though occasionally the compounds with the deficiency seemed a little paler. The pointed wing character was never exaggerated. The duplication 101 covers the poi alleles as it covers svr. Only once (for poi s) was it observed that the covering is not complete and that the poi s/Df-Dp flies were paler than normal.

As the break of the Bld translocation is very near to svr and the Bld phenotype is also one of pigment reduction, we tried to see a compound effect between Bld and the svr alleles, but did not succeed. Bld is already paler and tends to a trident rather irregularly. The compounds are not different and there is certainly no pointed effect. The same applies to Inversion  $y^{x^{b1}}$  with one break identical with that of Bld. But a quite unexpected result was obtained when these rearrangements were combined with bran (remembering that bran together with poi alleles produces blistering.) In the combination bran/T(Bld)1-2, poi/T(Bld)2→1 a soft blistered and Blond male, heterozygous for bran (tested), was obtained once. As bran could not be entered into the Bld second chromosome because it is located too near the Bld (2) break, only the heterozygous second chromosome could be tested. If it were obtained as bran/bran with Bld the effect might appear more clearly. The deficiency (1)Bld with bran in the second chromosome and poi opposite Bld was also not obtained.

But the yellow $^{x^{b1}}$  inversion gave a clear result.  $F_2$  from a cross  $y^{x^{b1}}$  bran segregates  $1/8$  bran/bran,  $y^{x^{b1}}$ , and these were soft blistered in addition to yellow. *In this case the second break of the inversion showed a position effect typical for the region of the right break together with the yellow position effect of the left break.* Another combination with a similar effect, namely,  $y-Inv$  with a dominant Bran, will be described below. The importance of these facts has been stressed in another paper (Goldschmidt, 1944).

The combinations of svr and the suppressor of sable were also tested. Though svr is different from the  $svr^{poi}$  alleles with respect to the sp suppression, we were surprised to find a very small combination effect with bran: bran/bran, svr flies are bran and pale, but in a large brood two females had a little blister near the wing tip. Also, the bran/bran,  $su-s$  combination was simply bran but had a tendency to soft spread wings, thus showing a poi character not visible in  $su-s$  alone.

All these facts show a rather remarkable situation. As a whole, the silver alleles, the sable suppressor (the others were not tested but may safely be assumed to behave similarly), the Blond-translocation break, and the identical right break of the yellow Inversion, act as a series of alleles. As the deficiency and duplication tests show, all the loci are located to the left of these breaks and to the right of the scute

TABLE 76  
PHENOTYPES IN THE SILVER REGION

Mutant or compound or combination	Body color	Trident	Wing shape	Suppressor action	Remarks
svr stock	Pale	....	Varying, poi	.....	pointed and soft wings always clear after extracting from crosses
w <sup>a</sup> svr stock	± pale	Present	Not poi	.....	pointed and soft wings always clear after extracting from crosses
All svr <sup>poi</sup> alleles	Pale	Very rare	poi	Supp. sp and s, not v....	svr <sup>poi</sup> eq ♂ show sometimes trident
su <sup>2-s</sup>	+ (= not pale)	....	Not poi	Suppr. s and v....	More poi with svr stock, less with w <sup>a</sup> svr
svr/ svr <sup>poi</sup>	Pale	....	± poi	.....	
svr/ svr <sup>pois</sup>	Pale	....	± poi	.....	
svr/ su-8	+	....	+	.....	Also other svr alleles
svr <sup>poi</sup> / su-8	+	....	+	.....	All other svr alleles haploid effect, no exaggeration; all covered by Dp. 101. Only svr <sup>poi</sup> dis <sup>5b</sup> /Df is lethal
svr/Df y-svr	Very pale	....	Not very poi	.....	
svr <sup>poi</sup> /Df y-svr	Very pale	....	Not very poi	.....	
svr <sup>poi</sup> s/Df y-svr	Very pale	....	Very poi	.....	
svr <sup>poi</sup> /Dp 101	+	....	+	.....	
Bld/+	Paler than + (Canton)	Frequent	+	.....	
Bld/ svr <sup>poi</sup> and svr	Like Bld	....	.....	.....	
I y <sup>px</sup> bl/Df y-svr	Lighter than homoz. Inv.	....	+	I y <sup>px</sup> bl no suppr. action.	
I y <sup>px</sup> bl/ svr	+	....	+	.....	Types soft blistered, etc.
bran/bran, poi/ poi	Pale	....	See poi alleles	.....	Once a soft blistered and Blond ♂ not def. for chr. 2
bran/T Bld, T Bld/ poi	.....	....	.....	.....	Between soft blist and bran blist (and yellow
bran/bran; I y <sup>px</sup> bl/ I y <sup>px</sup> bl	.....	....	.....	.....	Rarely blistered
bran/bran, svr	Pale	....	bran	.....	Many soft spread wings
bran/bran, su-8	+	....	bran	.....	

bands. But the minute rearrangements present in some of the alleles (see pp. 395 ff.) are located some bands to the right of these breaks. However, the phenotypical relations of all these alleles and compounds are, I might say, fractioned. *Su-s* has no allelic effect with *svr* and its alleles in regard to the character pointed wings and pale color; it is, however, allelic to the *svr<sup>poi</sup>* alleles in regard to *s* suppression. On the other hand, the *svr<sup>poi</sup>* alleles are *sp* suppressors (as are also some of the other unanalyzed suppressors), but they are not allelic to *su-s* in regard to *v* suppression present in the latter. The mutant *svr* is allelic to the *poi* alleles in regard to pale color and pointed wings, but not in regard to the suppressor action. The break of Blond has a related effect to paleness in *svr*, acting only on hair. The combination effect with *bran* (blistering) is extreme in some *poi* alleles, smaller in others, almost absent in *svr*, incipient in *Bld* (no *bran/bran* tested), and considerable in yellow Inversion. This picture parallels to a certain degree that described for the yellow or black or intermediate hair color and the different yellow body colors in *y* alleles, and might also be compared to some of the features described for the scute and achete phenotypes. We might express the facts in the following diagram.

Visible effects	Mutants					
	<i>su-s</i>	<i>su-v</i>	<i>svr<sup>poi</sup></i> alleles	<i>svr</i>	<i>Bld</i>	<i>y-Inv</i>
Suppression <i>s</i> .....	_____					
Suppression <i>sp</i> .....		_____				
Suppression <i>v</i> .....	_____					
Pale and <i>poi</i> .....			_____			
Blond hair .....					_____	
Combination effect with <i>bran</i> ..	—	?	_____	—	_____	_____

I consider these facts (as well as the corresponding ones for *y*, *ac*, *sc*) an indication that the concept of the corpuscular gene and its chemical changes in mutation does not cover the actual facts; that, moreover, a chromosome segment of a number of bands is the serial unit in some respects, and that all disturbances within that segment produce a series of multiple alleles. A part of this conception has recently been discussed in Goldschmidt, 1944; a more detailed analysis may yet be given.

*The arc region.*—The arc region seems to show features similar to those in the silver section. Though we assumed that the *bran* alleles are alleles of *arc*, we did not symbolize them as *a<sup>bran</sup>*, etc., because the facts do not seem so simple as this. The localization of *bran* put it at or near the *arc* locus. No crossover between the two was ever observed, and thus no combination of them was obtained. *Bran* and *plexus*, the latter nearest to *arc*, occurred simultaneously (by mutation) and were closely linked, though rare crossovers occurred just as between *a* and *px*. But the compound and deficiency tests did not give a simple result. Table 77 records a number of these tests for *bran* and some of its alleles. The compounds with *arc* and with the *arc* deficiency both indicate allelism. The *bran* alleles with no considerable phenotypic effect, such as *bran<sup>1</sup>*, *bran<sup>ap</sup>*, have little or no effect in the compounds. A remarkable case is *bran<sup>2</sup>*, which is a one-band deficiency lying inside the rather long *a px-Df*, as described by Bridges. One should expect the compound to be either lethal or at least of the *bran* type if not exaggerated. Actually, the compound hardly differs



from the a px deficiency over normal. (If, however, in a backcross the bb allele in the first chromosome of bran blist = bran<sup>a</sup>/bran<sup>a</sup>, poi<sup>aa</sup> is added to the compound, an extreme achi bb fly is produced in which the deficiency and bb effect upon the abdomen seem additive.) This is remarkable, as the compounds of bran<sup>a</sup> with bran (see list of phenotypes, table 74) have broad wings, and even the compound with arc is sometimes flapper-like. Certainly, these phenotypes do not fall into line as simply as in other multiple allelic series. Another group of remarkable facts is the following. We saw that many bran alleles give a combination effect with svr alleles,

TABLE 77  
PHENOTYPES IN THE arc REGION

Combine	Phenotype of wings
bran/a . . . . .	Broader than wild type, sometimes variation toward almost bran
bran <sup>dp</sup> /a . . . . .	Broader or normal
bran <sup>1</sup> /a . . . . .	Broader or normal
bran <sup>2</sup> /a . . . . .	Almost bran, once broad flapper-like
bran <sup>3</sup> /a . . . . .	+
Df(2)a px/+ . . . . .	Wings broader, some turned up, Minute characters
Df(2)a px/a px . . . . .	Wings like flappers, turned up (plexation only a little exaggerated), ♀ etched, few blist., all M
Df(2)a px/bran . . . . .	Variation from broader, part arched to real bran and flapper-like, most males scalloped at posterior edge; all M
Df(2)a px/bran <sup>dp</sup> . . . . .	Very broad, part arched; all M
Df(2)a px/bran <sup>1</sup> . . . . .	Almost normal wings; all M
Df(2)a px/bran <sup>2</sup> . . . . .	More like Df/+ than like Df/bran, very few nicked males
Df(2)a px/bran <sup>3</sup> . . . . .	arc-like; all M
Df(2)a px/bran <sup>4</sup> . . . . .	Almost normal wings, part of males scalloped

such as soft blistered, pointed blistered, etc. Homozygous arc with pointed has no such effect, and neither has the Df(2)a px/+ with pointed. But occasionally among Df(2)a px/+ flies without pointed, individuals are found with one dumpoid wing, thus showing a tendency toward a phenotype frequent in bran alleles and combinations with poi. In view of the length of this deficiency this fact may as well be based upon identity as upon a close linkage of the bran and arc loci. Thus, though I believe both to be arc alleles, I cannot prove it beyond doubt and therefore also hesitate to state that the one-band deficiency of bran<sup>a</sup> contains the arc locus. I suspect that we are dealing with a case comparable to that discussed for the svr locus, i.e., the presence of a number of bands with consequent arc or broadening effect caused by visible rearrangements or invisible mutants within the entire segment.

## 2. THE CONSTITUTION OF THE plexus blistered (px bl) STOCK

### a. INTRODUCTION

The mutants described thus far originated repeatedly, directly or indirectly, from our so-called plexus blistered stock. We shall see later that after outcrossing this stock or its derivatives a considerable number of other mutants appeared. An explanation for the process of mutation in the material upon which this paper is based therefore requires a knowledge of the genetical condition of the basic px bl

stock, which will be analyzed in more detail than otherwise would appear necessary. Actually, our lack of knowledge of the spontaneous process of mutation is caused, in part at least, by neglect of details which do not seem to offer any new information to the geneticist and which are shoved aside as mere repetitions of well-known facts. Therefore we feel obliged to present many data which might otherwise be easily branded as elementary and not worth mentioning.

As was reported above, the px bl stock, the basis of the present analysis of mutation, contains the mutant locus plexus from a pure standard stock from which the px bl stock was derived. The blistering and high-grade plexation were obviously in part the additional effect of some bs (blistered) allele. Within the stock the amount of plexation and blistering varied, and the genetical situation revealed by selection as well as by crossing experiments was not a simple one. When analysis was attempted, it turned out that the phenotypes of different combinations not only overlapped, but also were influenced by different modifiers as well as by external conditions. The difficulty was increased when it was found that the bs stock used for test crosses contained another allele which produced certain compound effects phenotypically identical with others based upon a bs allele in the px bl stock. The disentangling of these relations required a detailed morphological study of the phenotypes found in px bl, in the plexation-producing tester stocks, and in the different compounds and combinations appearing in backcrosses. As the details furnish the information on the genetical constitution of px bl, data which in themselves are not pertinent to our problem will be presented as a part of the analysis.

The plexus blistered line (abbreviated px bl, without any genotypical meaning in these symbols), the origin of which from standard px was described above (see p. 293), has remained constant for many years, apart from the features already reported. This means that in mass culture as well as in closely inbred brother-sister cultures the same types were always found. There is, however, a definite variation, most easily recognized by the blistering of the wings, which characterizes the stock. For example, in one series of generations of the stock the number of blistered flies may be very high, and in another generation they may be almost absent in some bottles. The details of this variation exclude environmental influences as sole cause. Obviously, a genetic situation is present which changes its equilibrium in the mass cultures. But in the end the population is more or less the same. As an example I may mention the fact that some of my stocks threw a high percentage of blistered flies for many years, and with such constancy that I always had blistered flies available for experimentation. During the year 1940 all these stocks changed to very low incidence of blistering without any known change occurring in the external conditions, though the high-grade plexation remained. Two sets of selections were started by breeding from blistered females and, where possible, from blistered males also. In one of these lines the old condition of the stock was restored after three generations; in the other, selection succeeded in going beyond that, many of the males being also blistered (see below). Obviously, then, the main condition for blistering is homozygous in the stock, but additional modifiers needed for high incidence may be selected or counterselected. Actually, also, lines without any blisters at all, not distinguishable from px, have been selected. Other conditions of the stock became visible only after outcrossing, which varied in the stocks in the course of time, as will be discussed below.

## b. DESCRIPTION

In general, this line may be described as typical high-grade plexus with more or less irregular occurrence of blisters upon the wing. A detailed analysis, however, shows the plexus formation not to be identical with that of the standard plexus mutant. As a matter of fact, the different laboratory lines of this mutant, though rather constant, are different from one another. It is not known whether different alleles or modifiers are involved. The lowest type known to me appears in a standard<sup>a</sup> combination with dumpy. This, however, is not due to a modifying action of dp, as a high plexation may be combined with dp and the low one separated from dumpy.

In the description, we shall use the following abbreviations and symbols (see plate 23), dispensing with the entomological terms. We mark the series of wing cells anterioposteriorly (called marginal, submarginal, first, second, third posterior cells) as I-V. An extra vein, or dot, or dash, or net, etc., in II means, therefore, a dash, etc., in the submarginal cell, etc. Correspondingly, we mark the longitudinal veins 1-5. A knot or an antler at 2 therefore means such a formation of extra veins attached to the second vein. The extra veins frequently unite with their point of attachment into a broad chitinous mass, sometimes strongly chitinized, sometimes not completely so. We call such a formation suggestive of a webfoot, a web. Cv is the posterior cross vein, to which extra veins or webs may be attached.

One of the most frequent extra veins is a dot, dash, or line in V, parallel to 5 near cv. It will be designated EV. If this EV elongates and finally forms a complete longitudinal vein, we call it a parallel vein. Frequently 4 and 5 branch off veins parallel to the wing margin. They may unite to form an arch vein or a periclinal vein.

The lowest px type shows only the following extra veins: a few branches of 2 into I toward the tip of the wing; a similar short branch from 2 into II; a short branch from cv into IV. A somewhat higher type of px, as isolated from a marked second chromosome stock, contains lines or net formation in I, sometimes dots in III. Frequently there is a dot or dash EV and a small antler formation attached to 5 near cv. A still higher plexus type, typified in a standard a px sp stock, is similar to the foregoing, but plus individuals have also more or less of the parallel vein in V and of the arch vein in IV. This last type corresponds with the original description of plexus in the Bridges-Morgan monograph (1919).

In the original plexus blistered line the lowest grades do not occur. The lowest type of plexation corresponds closely to the highest type in standard px as just described. But in numerous individuals a considerable web formation is attached to 5 near cv. More frequent is a higher type with a considerable web formation which unites the attachment of the arch vein to 5 with the attachment of the parallel vein in V. This web is broad and elongated. These two types, generally called low and medium plexus, are characteristic for populations with few individuals showing blistered wings.

In populations with much blistering a still higher type is prevalent (strong plexus). The web at 5 is extremely long and broad, arch vein and parallel vein are complete, and even branch into a network. The antler formations at 2 and 3 are also expanded into broad webs.

<sup>a</sup> "Standard" = derived from original Columbia and, later, Pasadena stocks.

Blistering may occur in these stocks, on one or both wings. The blister appears above the web at 5 and extends over a considerable part of the wing, more so when both wings are blistered. The blisters found in the lower plexus types at the same point, though more rare, are correspondingly less extreme. There exist still more extreme types of plexus formation which, however, do not occur in the pure px bl stock (see below).

The foregoing description applies to females. Males generally show the same types, but all features are less extreme; one might say they are one class below the female condition. Blisters are usually rare in the males, though lines with high incidence occur. The sex ratio is almost normal in the line (see below).

### C. THE PHENOTYPE OF SECOND-CHROMOSOME MUTANTS AND DEFICIENCIES

#### PRODUCING PLEXATION

Analysis of px bl requires a knowledge of the phenotypes of the tester mutants of a plexus-like type, which will be presented in this section.

*Plexus (px).*—The mutant px is available in lines with a different degree of phenotypic effect (see description below). No special inquiry has been made with respect to whether these represent multiple alleles or modifier effects. A low-grade plexus, as found in the px dp stock, does not show much extra venation. There is a posterior branch of the cross vein of different length; there is a dot, dash, or line in the marginal cell, and the third longitudinal vein carries posteriorly a more or less pronounced antler formation. Furthermore, the marginal ends of the veins are bent and sometimes branch into a delta. In the higher grades of px all these features are more strongly emphasized, the extra vein in the marginal cell becomes attached to the second longitudinal vein and forms branches, and a branch from the fifth longitudinal vein unites with a branch from the fourth vein, thus forming what we call the periclinal or arch vein. The attachment of the periclinal vein to the fifth longitudinal may become transformed into a network or a broad chitinous mass (web). In the third posterior cell a dot or dash appears. Plexus, which is registered as a recessive mutant, possesses a considerable amount of dominance. All crosses with wild type showed at least a dot or dash in the marginal cell in up to 75 per cent of the  $F_1$  individuals. Whenever px enters into any heterozygous combination, this dominance effect will be found in a number of individuals and can be used as a marker. Plexus opposite a deficiency is not much exaggerated.

*Blistered (bs).*—The standard blistered (bs 107.3) which we received from Pasadena has remained rather constant in our cultures. The phenotype varies from perfectly normal flies through a series showing a small dot in the fifth posterior cell near the cross vein, to flies with a more or less long dash at this point, or a small antler-like structure more or less attached to the fifth longitudinal vein. We made frequent statistical checks of the conditions in stocks and pair cultures and found a certain fluctuation with a mean near the type of beginning antler formation. For crossing we always preferred the plus type with antler, which, as we shall see, was sometimes a compound of two bs alleles. Only once did we find a bs fly with plexus formation on the wing; the offspring, however, was not different from that of other flies. A pure bs fly with blisters on the wings, such as had been the type of the original mutant, was never found, and this at different external conditions, warm and cold. But we shall have to mention below certain bs alleles which produce a

definite type of blister. (These details are needed because this, like so many other stocks, no longer shows the characters given in the original description.) The bs of the line here used is, like px, an incomplete dominant; about 60 per cent of the heterozygotes with wild (Oregon) are normal, the rest varying from a small dot in the fifth cell to the complete antler. Whereas up to 20 per cent of normals were found in the pure bs broods, this means a considerable dominance. Once a single cross showed complete dominance of the antler in all individuals. (This was found prior to the discovery of the second allele and therefore was not tested for it.) The heterozygous dominance effects as well as the homozygous effects of bs and px are simply additive if present in the same individual.

*Balloon (ba).*—A third locus with a plexus effect is balloon (II.107.4). This locus was originally (see Bridges-Morgan monograph) supposed to produce a plexus effect with inflated wings. Among the stocks, all received from Columbia and later from Pasadena and in repeated sets, over many years, an individual was never found corresponding to the original description, whatever the external temperature. As a matter of fact, for years the balloon lines, which are supposed to be more conspicuous at lower temperatures, showed nothing but small and rare extra veins at whatsoever temperature and were practically useless. Some time ago, the same stock bottles bw ba sp kept in the same external conditions were found to contain exclusively a very distinct type which completely fits the original description but for the total absence of blisters under good conditions. (Blisters appear in old bottles when the flies become very small.) This type has since bred true irrespective of external conditions, and segregates typically from crosses. It is characterized by an extreme plexus formation which contains all the elements described for former combinations but with a somewhat different pattern. It shows in considerable degree all the elements of the plexus pattern. The antler formation in the fifth cell involves the whole end of the fifth longitudinal vein and is, in most cases, found as a broad chitinous mass. The periclinal vein is more or less complete, though nearer to the wing margin than in the former cases. The parallel vein is found in the plus individuals also. Near the ends of the third and fourth veins a considerable antler formation is found and long extra veins are located in the marginal cell (I), which is very characteristic. There is an inclination toward the formation of a network of extra veins in the submarginal (II) and third posterior cell (V) in the extreme plus individuals. The minus individuals show all these elements only slightly expressed.

We worked for some time with this balloon stock before strange crossover results suggested a check. (I emphasize again that at this time the stock had the phenotype as originally described.) Crossover tests with wild type as well as with marked stocks resulted in a practically free segregation of the plexus formation, considered to characterize ba, from bw, and sp, the last-named only 4 units from ba. An example of this is shown in table 78.

This indicated that the plexation, identical with the original phenotype, which had reappeared in the stock, was based upon a locus at the other end of the second chromosome. Therefore the locus net was suspected to be present in the stock. Crosses with the mutant net produced a plexus type different from both the net and the ba stock. From a cross with markers (as well as the one reported; table 78), individuals were isolated which showed the complete plexation without bw sp (see class 4 of table 78) and therefore were suspected to be the net crossovers. Crossed

to net, they produced all net flies, part of them blistered and part dark-colored. Actually, then, the change in the ba stock was the appearance of a net allele and its spread in the stock. The same net allele must have been present in the original stock. The compound tended to blistering (possibly based upon the additional presence of ba) and a constant net blistered stock was extracted. The dark color which appeared in one-fourth of the offspring from net  $\times$  crossover net and of bw, sp, ba, and which was associated with rough eyes, stocky build, and bristle abnormalities, turned out to be an ebony allele. Further details do not belong here, but it ought to be mentioned as a remarkable fact that ebony was originally discovered in ba stock (by Miss Wallace) one of many similar instances which point to mutation as not a chance phenomenon.

*The Plexates (Df(2)Px).*—The fourth plexus-producing type which has been useful in analyzing the px bl stock is the Plexates. Bridges studied two deficiencies

TABLE 78  
(bw sp ba  $\times$  Ore)  $\times$  bw sp ba

	♀	♂	
1. +.....	60	69	
2. bw sp ba. ....	58	66	(as in stock)
3. bw sp.....	36	50	
4. ba (= px).....	61	67	(as in bw sp ba stock)
5. bw.....	..	2	
6. sp ba.....	1	1	
7. bw ba.....	..	2	
8. sp.....	2	2	

involving the bs and ba loci, which he called Plexates because they produce a kind of plexus effect in heterozygous condition. They are lethal when homozygous.  $Px^2$  is a deficiency for blistered and balloon, and  $Px^1$  includes also speck.

The effect of  $Px^2$  in heterozygous condition,  $Px^2/+$ , is not like one of the bs effects. There is a more or less pronounced antler formation in the third cell (V), varying from a little to a real antler formation. Also, there is a posterior branch to the cross vein and frequently a beginning of an antler formation attached to the third longitudinal vein in the submarginal cell. Moreover, extra veins are found in the marginal and the submarginal cell, which probably are the effect of the ba deficiency since ba heterozygotes show the same phenomenon (but in the presence of net/+) (see above). The compound  $Px^2/bs$  shows the expected exaggerated effect. All parts of the  $Px^2$  pattern are more pronounced, and most individuals exhibit a part of the parallel vein as before. Sometimes a very small pearl-like blister appears on top of the broadened antler. But in some cultures all females have a considerable blister. Other types, based upon different alleles, will be described later. Males are not blistered. There is a certain range of variation and the sexual difference is encountered as always. (It has to be emphasized again that, in almost all combinations involving any type of plexus formation as well as blistering, the males exhibit a grade which in plus individuals nears the minus grades of females.)

$Px^1$  contains, according to Bridges, the speck locus besides bs and ba, as can be easily checked. It is a longer deficiency than  $Px^2$ . It is also lethal when homozygous.

TABLE 79

PHENOTYPES OF HOMOZYGOTES, HETEROZYGOTES, COMBINATIONS, AND COMPOUNDS INVOLVING WING PLEKXATON  
(The table gives the modal phenotype of females under conditions as nearly identical as possible and, where possible, from inbred lines  
For explanation of abbreviations, see p. 404.)

Genotype	Extra venation in cell					Parallel vein	Peri- clinal vein	Antler web		Net in	Bilatered		Ex- treme bl	Sack
	I	II	III	IV	V			At 5	Near av		One wing	Both		
1. px/px —	+	+	+	+	+	±	+	+	+	..	..	..	..	..
2. px/px +	+	+	+	+	+	±	+	+	+	..	..	..	..	..
3. px/+	+	+	+	+	±	±	+	±	+	..	..	..	..	..
4. bs/bs.	..	..	..	..	±	±	..	±	..	..	..	..	..	..
5. bs/+	..	..	..	..	±	±	..	±	+	..	..	..	..	..
6. bs <sup>2</sup> /bs <sup>2</sup>	..	..	..	..	±	±	..	+	+	II	..	..	..	..
7. bs/ba (incl. net)	+	+	..	+	..	±	+	+	+	I, II, V	..	..	..	..
8. bs/+	+	+	..	+	..	±	..	+	+	..	..	..	..	..
9. Px <sup>1</sup> /+	+	+	..	+	..	±	..	+	+	..	..	..	..	..
10. Px <sup>2</sup> /+	+	+	..	+	..	±	..	+	+	..	..	..	..	..
11. px bl stock.	±	±	..	±	±	±	±	±	±	..	±	±	..	..
12. px bl/+	+	..	..	±	+	±	±	±	±	..	±	±	..	..
13. px +/+ bs = typ 3 + 5.	..	..	..	±	+	±	±	±	±	..	±	±	..	..
14. px +/+ ba = type 3 + 8.	..	..	..	±	+	±	±	±	±	..	±	±	..	..
15. bs +/+ ba = type 5 + 8.	..	..	..	±	+	±	±	±	±	..	±	±	..	..
16. px +/+ Px <sup>2</sup> = type 3 + 10.	..	..	..	±	+	±	±	±	±	..	±	±	..	..
17. bs/Px <sup>2</sup>	+	+	..	±	±	±	±	±	±	..	±	±	..	..
18. bs/Px <sup>1</sup>	+	+	..	±	±	±	±	±	±	..	±	±	..	..
19. bs/Px <sup>2</sup>	+	+	..	±	±	±	±	±	±	..	±	±	..	..
20. bs/Px <sup>1</sup>	+	+	..	±	±	±	±	±	±	..	±	±	..	..
21. px bl/px	±	±	±	±	±	±	±	±	±	I, II, V I, II, V	+	+	..	..
22. px bl/bs	+	+	±	±	±	±	±	±	±	..	+	+	..	..
23. px bl/ba.	+	+	±	±	±	±	±	±	±	..	+	+	..	..
24. px bl/Px <sup>1</sup>	+	+	±	±	±	±	±	±	±	I, III I, III	+	+	+	±
25. px bl/Px <sup>2</sup>	+	+	±	±	±	±	±	±	±	I, III I, III	+	+	+	±
26. Bid Def/+	+	+	±	±	±	±	±	±	±	I, II, V	+	+	+	±

TABLE 79—(Continued)

Genotype	Extra venation in cell					Parallel vein	Peri-olinal vein	Antler web		Net in	Blistered		Extram bl	Sack
	I	II	III	IV	V			At 5	Near cv		One wing	Both		
27. Bid Def/px bl.....	..	Like 26	..	..	..	..	..	..	..	..	..	..	..	Almost
28. Bid Def/bs.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
29. px bs/px bs = type 2 + 4.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
30. px ba/px ba = type 2 + 7.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
31. net.....	+	+	+	+	+	..	+	+	+	..	+	+	..	..
32. px bs <sup>p</sup> /+ bs <sup>p</sup> .....	+	+	..	..	..	..	+	+	+	..	+	+	..	..
33. px bs/+ Px <sup>s</sup> .....	+	+	..	..	..	..	+	+	+	..	+	+	..	..
34. bs <sup>p</sup> /Px <sup>s</sup> .....	+	+	..	..	..	..	+	+	+	..	+	+	..	..
35. bs <sup>s</sup> /bs.....	+	+	..	..	..	..	+	+	+	..	+	+	..	..
36. bs <sup>s</sup> /px bs <sup>p</sup> .....	+	+	..	..	..	..	+	+	+	..	+	+	..	..
37. px +/+ bs <sup>s</sup> .....	+	..	..	..	..	..	+	+	+	..	+	+	..	..
38. bs <sup>s</sup> /Px <sup>s</sup> .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
39. bs <sup>bl</sup> /px bl.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
40. bs <sup>bl</sup> /Px <sup>s</sup> .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
41. bs <sup>bl</sup> /bs <sup>bl</sup> .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
42. bs <sup>bl</sup> /+.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
43. px bs <sup>p</sup> /px +.....	±	±	..	±	..	..	±	±	±	..	±	±	..	..
44. px bs <sup>bl</sup> /px +.....	±	±	..	±	..	..	±	±	±	..	±	±	..	..
45. px bs <sup>p</sup> /px bs <sup>p</sup> .....	±	±	..	±	..	..	±	±	±	..	±	±	..	..
46. px bs <sup>p</sup> /px bs <sup>bl</sup> .....	±	±	..	±	..	..	±	±	±	..	±	±	..	..
47. px bs <sup>bl</sup> /px bs <sup>bl</sup> .....	±	±	..	±	..	..	±	±	±	..	±	±	..	..
48. bs <sup>bl</sup> /Blond Def almost lethal.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
49. bs <sup>p</sup> /+.....	..	±	..	..	±	..	..	..	..	..	..	..	+	..
50. bs <sup>p</sup> /bs <sup>p</sup> .....	..	..	..	..	..	..	..	..	..	..	..	..	between	..
51. bs <sup>p</sup> /bs <sup>bl</sup> .....	..	..	..	..	..	..	..	..	..	..	..	..	..	..
52. bs <sup>p</sup> /Px.....	..	net	..	..	..	..	..	..	..	..	..	..	..	..
53. bs <sup>s</sup> /bs <sup>s</sup> .....	..	..	..	..	..	..	net	..	..	..	..	..	..	..
54. bs <sup>s</sup> /bs.....	..	..	..	..	..	..	..	±	..	..	..	..	..	..



Heterozygous  $Px^1/+$  resembles  $Px^2/+$  but has a somewhat stronger effect. The antler in the posterior third cell may be broadened to a chitinous mass, and the extra veins in the marginal and submarginal cell are better developed. The phenotypic action, then, corresponds to one of a higher allele.

The compound  $bs/Px^1$ , again, is similar to the one with  $Px^2$  but is of a little higher grade; the blisters on top of the expanded antler are more frequent and a number of individuals have one wing really blistered.

Very typical are the compounds of  $ba$  (stock containing net) with the *Plexates*.  $ba/Px^2$  shows the expected exaggeration effect for  $ba$ . This consists in a very strong expression of all the features of venation characterizing  $ba$  with net. It becomes especially visible in the extra veins of the submarginal and marginal cells, which form an extended and dense network; also in the third posterior cell the extra veins form nets. These characters are specific for the net containing  $ba$ , though their beginnings appear also in the  $bs$  series. One might actually describe these  $ba$  features as an extreme type of  $bs$  action, localized at certain points. Furthermore, many individuals of this compound are blistered. The compound  $ba/Px^1$  is very similar, only still more extreme, and almost all females are blistered. This, again, is rather remarkable;  $ba$  separated from the net allele in our stock has no phenotypic effect and is useless, as stated before. But the exaggeration visible in the  $Px/ba$  compound is an exaggeration of the features produced by the net allele at the other end of the chromosome. This means that net acts not only as a producer of the net phenotype, but simultaneously as an enhancer for the otherwise subthreshold  $ba$  action. The exaggeration of the  $ba$  effect opposite a deficiency is simultaneously an exaggeration of the net effect located far away!

Here, then, as before, the combinations of a deficiency with  $bs$  and  $ba$  causing exaggeration of the plexation produce blistering as a part of more extreme plexation.

*Blond* ( $T(1,2)Bld$ ).—Another deficiency in this region which causes a plexus formation is *Blond*(2) deficiency, which removes the tip of II and thus is also deficient for  $sp$   $bs$  and  $ba$  (but is duplicated for the tip of X). The plexus type is very similar to the one described for  $ba + net$ , but in addition the wings are spread though not blistered. This deficiency in compound with  $bs$  is not very much exaggerated, but is exaggerated with other  $bs$  alleles (see below).

All these types and some not mentioned, as well as those to be discussed in succeeding sections, are listed for easy reference in table 79.

#### d. THE $Px$ AND $bs$ LOCI IN $Px\ bl$

*Crosses with  $bs$  and the *Plexates*.*—As was stated before, the analysis has been complicated by the fact that standard  $bs$  is frequently heterozygous for a higher allele, called here  $bs^{b1}$ , which is very poorly viable in homozygous state and has a compound effect with  $bs$  within the range of variation of the  $bs$  phenotype. Also, some of the compounds involving the four alleles  $bs^{pp}$  and  $bs^p$  from  $Px\ bl$ , and  $bs$  and  $bs^{b1}$  from  $bs$  stock, are phenotypically alike. This led to many detours. If  $Px\ bl$  is crossed to  $bs/bs$  and  $Px\ bl$  is from a balanced stock (to be described), namely,  $Px\ bs^{pp}\ 1/Px\ bs^p\ 1^+$  ( $1^+$  being a lethal balancing  $bs^p$  against homozygous lethal deficiency  $1$ ),  $F_1$  segregates into the distinguishable though somewhat overlapping types,  $bs^{pp}/bs$  and  $b^p/bs$ . If  $Px\ bl$  comes from a selected low stock lacking  $bs^{pp}$  and  $1$  as well as  $1^+$ , all  $F_1$  are alike, that is,  $bs/bs^p$ . Both of the  $Px\ bl$  types might also have

been crossed with a bs invisibly heterozygous for bs/bs<sup>b1</sup>. In the first case, four types ought to appear in F<sub>1</sub>, namely, bs<sup>pp</sup>/bs, bs<sup>pp</sup>/bs<sup>b1</sup>, bs<sup>p</sup>/bs, bs<sup>p</sup>/bs<sup>b1</sup>. With the low stock the segregation would be into bs<sup>p</sup>/bs and bs<sup>p</sup>/bs<sup>b1</sup>. It was soon found that it is easier to distinguish the types obtained when the Plexates (1 and 2) are crossed into the stocks, which again led to analysis of the phenotypes produced in such crosses.

Table 80 contains some results of crosses px bl × bs. The stock px bl low (no. 11) is one in which the bs mutants had been selected out (px/px remaining). The pheno-

TABLE 80  
PHENOTYPES OF F<sub>1</sub> FROM DIFFERENT px bl × bs

No.	Cross	♀		♂		Only EV, no antler	
		± antler	antler and web	± antler	antler and web	♀	♂
837	px bl × bs.....	54	..	37	..	..	..
844	px bl × bs.....	15	..	8	..	..	..
845	px bl × bs.....	88	..	29	..	..	..
857	px bl × bs.....	85	..	39	..	..	..
841	px bl blist × bs.....	50	..	22	..	..	..
834	bs × px bl.....	46	..	..	..	..	33
833	bs × px bl.....	..	..	..	..	47	45
835	bs × px bl.....	..	..	..	..	48	37
847	px bl low × bs.....	..	..	..	..	59	44
851	bs × px bl low.....	..	..	..	..	42	23
852	bs × px bl low.....	..	..	..	..	27	43
838	px bl × bs.....	15	31	22	7	..	..
843	px bl × bs.....	29	23	25	3	..	..
854	px bl × bs.....	11	11	4	4	..	..
855	px bl × bs.....	51	43	16	15	..	..
638	px bl × bs.....	22	34	20	24	..	..
639	px bl × bs.....	9	15	12	12	..	..
641	px bl × bs.....	1	12	3	1	..	..
642	px bl × bs.....	44	31	20	24	..	..
840	px bl blist × bs.....	29	21	22	11	..	..
846	px bl low × bs.....	40	15	..	..	..	39
849	px bl low × bs.....	8	9	..	..	..	35
853	bs × px bl low.....	18	17	..	..	..	34

types EV antler, web have been explained above. The table shows four types of F<sub>1</sub> results: (1) no segregation, and both sexes with antler near the cross vein; (2) no segregation, but both sexes of a lower type; one cross, 834, combines both types in the ♀♀ and ♂♂, respectively; (3) segregation into ½ antler, ½ web in both sexes; (4) the same segregation in ♀♀, but all ♂♂ low. Nos. 2 and 4 occurred six out of eight times when px bl low was involved. These results agree with the assumption that most of the parents from px bl were heterozygous bs<sup>pp</sup>/bs<sup>p</sup>, which produces with bs the compounds bs<sup>pp</sup>/bs = web and bs<sup>p</sup>/bs = antler (group 3). A number (group 1) had been bs<sup>p</sup>/bs<sup>p</sup>, and F<sub>1</sub> was uniformly antler (once the expressivity was lower in the males and twice in both sexes). Selected px bl low = px without bs gave with bs only the bs/+ effect = group 3. Only in group 4 was the bs parent obviously heterozygous for bs<sup>b1</sup>. By chance, no combination bs<sup>pp</sup>/bs<sup>p</sup> × bs/bs<sup>b1</sup> was made, which

would have been easily recognized because  $Df/bs^{b1}$  is a very extreme type called "almost sac" (see below).

We tried to prove these formulations by  $F_2$  and backcrosses breeding from the different  $F_1$  types. The results agreed with expectation, but for more transgressing variation toward EA and +, probably resulting from recombination of modifiers when the  $F_1$  bs or antler types were bred. But when the web types from  $F_1$  were bred, the segregation was such that no classification came near the simple expected ratios. Of course web might have been either  $bs^{np}/bs$  or  $bs^{b1}/bs^p$  (and, in addition,

TABLE 81  
px bl DIFFERENT TYPES  $\times$  Cy/Px<sup>2</sup>

No.	px bl mother type	Phenotype $F_1$ (not Cy)
2497	PV, EV.....	EV. Only a few PV. Many ♀ few ♂ blist. No segregation visible
2498	PV, EV.....	EV. Only a few PV. Many ♀ few ♂ blist. No segregation visible
2499	PV, EV.....	Both sexes about $\frac{1}{2}$ blist, otherwise as before
2501	PV, EV, bl.....	Like 2497
2502	PV, EV, bl.....	Like 2497
2503	PV, EV, bl.....	Like 2499
2513	PV, Pa V, W.....	Like 2497
2504	PV, Pa V $\pm$ bl.....	Like 2499, but more PV
2505	PV, Pa V $\pm$ bl.....	Clear segregation of two types ♀ and ♂: $\frac{1}{2}$ as before with or without blisters, $\frac{1}{2}$ with extreme types of all veins and both wings extremely blistered
2507	PV:px, Pa V $\pm$ , W..	Like 2505
2508	PV:px, Pa V $\pm$ , W..	Like 2505
2509	PV:px, Pa V $\pm$ , W..	Like 2505
2510	PV, Pa V, W, bl....	Like 2505
2514	PV, Pa V, W, bl....	Like 2505
2512	PV, Pa V, W, bl N.	All ♀ ♂ of the extreme type as before

Legend: PV = periclinal vein  
 EV = Extra vein, third posterior cell  
 bl = blistered  
 Pa V = Parallel vein (— = minus type, + = plus type, no sign = complete)  
 px = Much plexus formation in cells  
 W = web-like broadening where V, cross vein, and Pa V join  
 N = net formation at 5th vein

(The other constant features of px bl, such as extra veins in I, antler at 3, not marked)

$bs^{np}$  and  $bs^p$  were or were not linked with lethals), but even then the results could not have been classified clearly. Hence we resorted to introducing the Plexates because the exaggeration effect of these deficiencies would produce clearer differences between the classes. On this occasion the presence of  $bs^{b1}$  was discovered, since  $bs^{b1}/Px$  shows the extreme type sac.

We must first find the phenotypes of the possible compounds. Those of + or  $bs/Px^1$  or  $Px^2$  have been described above. In crossing px bl from nonselected stock with the Plexates, we realize that px bl may be the balanced individuals  $bs^{np} 1/bs^p I^1$ , or  $bs^p/bs^p$ . Actually, these can be distinguished phenotypically, as will be shown below, though there is a certain amount of transgression. The more extreme plexation and blistering indicate the compound with the high allele (plus homozygous px); the less extreme grades lack this allele. Table 81 reports upon the phenotypes of a series of crosses of females px bl, taken simultaneously from an inbred stock, with  $Cy/Px^2$

males. Only the major features of the phenotypes of P and  $F_1$  have been recorded. In analyzing this table it must be kept in mind that  $px/px \times Px^2$  shows nothing but the ordinary heterozygous effects of both. Everything beyond this (see description, p. 409) is at least the additional effect of the exaggerated *bs* locus in  $px\ bl$ . The  $px\ bl$  females which were crossed were different in the details indicated in the table. In a general way, we have a lower group without much parallel vein (which is an increased EV) and with maximally an antler formation in the third cell near the cross vein. In the second higher group this is replaced by a fairly large, web-like structure. The parallel vein increases in length. One extreme individual (2512) has also a net formation in different cells. The  $F_1$  results correspond very closely to this grouping, with one overlapping case (no. 2505). The first group of females produces a lower type of  $F_1$  compound. The EV or antler is exaggerated to a web (less so in males, as always); there is a considerable plexus formation in the second cell,

TABLE 82  
BLISTERING IN  $px \times Px^2$

♀		♂	
not blist	blist	not blist	blist
4	12	16	2
4	30	3	17
6	35	13	16
18	11	31	2

and a varying number of blistered individuals, usually more females than males. But sometimes rather exactly  $\frac{1}{2}$  of both sexes are blistered. The blistered or not blistered condition of the mother obviously has nothing to do with the  $F_1$  blistering, which we found also in other compounds with  $Px^2$ .

In this table the somewhat varying percentage of blistering has not been recorded in detail. However, this was done in some other crosses and a few examples are listed in table 82. Obviously, the mother was  $bs^p/bs^p$ , and the type described for  $F_1$  is  $bs^p/Px^2$  (plus  $px/+$ ). It is much more plexated and blistered than  $bs/Px^2$ , thus indicating that  $bs^p$  is a higher allele.

The second, higher plexus group of  $px\ bl$  mothers produces a clear segregation into  $\frac{1}{2}$  females and males as before, blistered or not blistered, blistered meaning a small blister above the web in one of the wings, not the type of extensive blister present in the  $px\ bl$  line. The other half consists of females and males with an extreme plexus formation, i.e., all the elements in their maximal expression and, in addition, both wings heavily blistered. There is, finally, the very extreme mother of the cross 2512 which produced offspring exclusively of the last extreme type!

This looks as if  $px\ bl$  of the higher type had been heterozygous for two different *bs* alleles. It is hardly to be expected that one of them was a deficiency, as a viable homozygous deficiency of such size is very improbable. Otherwise, there was no evidence of a lethal class ( $Cy$  : not  $Cy$  = 2 : 1), but this might be meaningless because the  $Cy$  class was always much smaller in this series.<sup>20</sup> The test would be to backcross the different types to  $Px^2$ . Many reciprocal backcrosses to  $Px^2/Cy$  were therefore

<sup>20</sup> We shall return, below, to this point when discussing the visible deficiency linked with  $bs^{pp}$ .

made. As the  $Px^s$  deficiency is lethal when homozygous, the backcross can yield only the parental  $F_1$  type in the not Cy class, provided only the second chromosome is involved. Table 83 presents the results.

TABLE 83  
HEREDITY OF TYPES OF FLEXATION

No.	Cross	$F_1$	$RF_2$
2553	$Px^s \times 2504$ type (not blist)	all low (blist or not)	$\varnothing \frac{1}{2}$ like $F_1$ , $\frac{1}{2}$ extreme
2555	$Px^s \times 2499$ type (not blist)	all low (blist or not)	$\frac{1}{2}$ like $F_1$ , $\frac{1}{2}$ very low-grade px
2558	$Px^s \times 2498$ type.....	all low (blist or not)	Like $F_1$ but mostly blist
2563	$Px^s \times 2513$ type.....	all low (blist or not)	Like $F_1$
2564	$Px^s \times 2502$ blist.....	all low (blist or not)	Like $F_1$
2577	$Px^s \times 2497$ blist.....	all low (blist or not)	Like $F_1$
2565	$2499$ blist $\times Px^s$ .....	all low (blist or not)	Like $F_1$
2569	$2498$ blist $+$ $\times Px^s$ .....	all low (blist or not)	Like $F_1$
2570	$2502$ type $\times Px^s$ .....	all low (blist or not)	Like $F_1$
2571	$2497$ blist $+$ $\times Px^s$ .....	all low (blist or not)	Like $F_1$
2573	$2502$ extr $\times Px^s$ .....	all low (blist or not)	$\varnothing$ about $\frac{1}{2}$ extr, $\sigma^7$ only few extr
2575	$2513$ blist $- \times Px^s$ .....	all low (blist or not)	Like $F_1$
2591	$2497$ blist $+$ $\times Px^s$ .....	all low (blist or not)	$\frac{1}{2}$ like $F_1$ , $\frac{1}{2}$ very low grade
2597	$2503$ blist $- \times Px^s$ .....	all low (blist or not)	$\frac{1}{2}$ like $F_1$ , $\frac{1}{2}$ very low grade
2599	$2498$ blist $- \times Px^s$ .....	all low (blist or not)	Like $F_1$
2596	$2503$ blist $- \times Px^s$ .....	all low (blist or not)	Almost all very low-grade px
2559	$Px^s \times 2505$ low.....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	Like father
2562	$Px^s \times 2505$ low.....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	Like father
2579	$Px^s \times 2514$ extr.....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing \frac{1}{2}$ extr, $\frac{1}{2}$ not; $\sigma^7$ extr px but not blist (like $\frac{1}{2} \varnothing$ )
2580	$Px^s \times 2507$ extr.....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing \frac{1}{2}$ extr, $\frac{1}{2}$ not; $\sigma^7$ extr px but not blist (like $\frac{1}{2} \varnothing$ )
2581	$Px^s \times 2505$ extr.....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ 2 not extr: 1 extr, $\sigma^7$ extr px but not blist (like $\frac{1}{2} \varnothing$ )
2568	$2505$ low blist $- \times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	Like mother
2574	$2508$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	*Many both wings blist, no extremes
2582	$2507$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ 2 not extr: 1 extr, $\sigma^7$ not extr
2584	$2507$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing \frac{1}{2}$ extr, $\sigma^7$ only few
2585	$2507$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ only few extr, $\sigma^7$ all low <sup>a</sup>
2587	$2507$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	About $\frac{1}{3}$ extr <sup>a</sup>
2588	$2507$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ about $\frac{1}{3}$ extr, $\sigma^7$ only few <sup>a</sup>
2589	$2505$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ about $\frac{1}{3}$ extr, $\sigma^7$ only few <sup>a</sup>
2590	$2505$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ about $\frac{1}{4}$ extr, $\sigma^7$ none, and some very low <sup>a</sup>
2594	$2505$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ about $\frac{1}{4}$ extr, $\sigma^7$ a few <sup>a</sup>
2595	$2505$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ about $\frac{1}{4}$ extr, $\sigma^7$ a few <sup>a</sup>
2598	$2514$ extr $\times Px^s$ .....	$\frac{1}{2}$ low, $\frac{1}{2}$ extr.....	$\varnothing$ about $\frac{1}{4}$ extr, $\sigma^7$ a few <sup>a</sup>
2572	$2512$ extr $\times Px^s$ .....	All extr.....	$\varnothing$ only few extr, $\sigma^7$ none (small numbers)

\* In these broods the mothers probably were not virgins, the ratios are therefore not relevant.

This table shows at once that something besides the second chromosome is involved in the  $F_1$ . A number of the  $RF_2$  resemble  $F_1$ , showing that here only the bs locus from px bl was involved, producing an exaggeration with the deficiency, the plexus, and partly blistered type, as described. But there are also a few different results. Three times (nos. 2555, 91, 97) only one-half of the individuals were like

the  $F_1$ ; the other half show only an extra vein and a branch at a cross vein and a short extra vein in I or II, these together being the type of plexation found in  $px\ bl \times wild$  or in  $Px/+$ . A few individuals of this type occurred also in other cases. It turned out that these were  $Cy/Px^1$  flies in which  $Cy$  did not show phenotypically.

Still more unexpected is the case of 2553, where one-half of the  $R\ F_1$  females (only one male) show the extreme blistered type not present in  $F_1$ . This indicates that a recombination with another chromosome containing an enhancer is responsible for this type. The same is borne out by the crosses involving the extreme type. The latter never bred true, as it ought to if it is based upon the  $bs$  locus alone, but segregated into extremes and not extremes, the latter being high-grade plexus but not blistered. The ratios which occur in the females are either 1 not extreme : 1 extreme or 2 not extreme : 1 extreme. Looking over these crosses, we realize further that no extreme males and few females are produced when the father is the  $F_1$  male, i.e., the X chromosome of the  $R\ F_1$  male is derived from  $Px^2$  stock. However, extreme males

TABLE 84  
( $px\ bl \times Px^2$ )<sup>2</sup>

No.	♀			♂			
	$px^+$ blist	extr $px\ bl$	$\pm$ web	$px$	$px\ bl$	extr $px\ bl$	antler-web
2670-71, 2323-26.	229	281	12	247	119	85	24

do appear in the reciprocal cross, though in small numbers. It is possibly the X chromosome from  $px\ bl$  which is responsible for this effect, and the females may be heterozygous or homozygous for the condition. But it is also possible that a dominant condition at the other end of the second chromosome is responsible and is removed in  $F\ R_2$  from  $bs$  by crossing over.

But one difficulty still remains: cross 2553, where one-half extreme offspring was sired by a low male from low  $F_1$ , which is supposed to have had neither the X chromosome with the condition for extreme blistering nor the assumed second-chromosome condition. No interpretation can be offered. The whole situation does not lend itself to a simple interpretation, beyond the statement that other loci are involved.

We assumed that in all these cases only  $bs^p$  was present, because  $bs^{pp}$  is associated with a deficiency inside  $Px^2$ . However, even if  $Px^2/bs^{pp}$  Df were viable and the extreme type, the result would not be any clearer. It may be added, finally, that in the  $bs$  situation, whatever it is, no other neighboring loci were involved. Many were tested but gave negative results, as has already been reported for one of them, namely, balloon. Thus we come to the conclusion that the  $bs^p$  allele in  $px\ bl$  is a higher allele of  $bs$  and that its effects are subject to considerable enhancement by modifiers in other chromosomes.

The phenotypes of  $bs^p$  homozygous without  $px/px$  could be found by crossing over between  $px$  and  $bs$ , which simultaneously removed the lethal linked in the original stock with  $bs^p$ . The clearest case was obtained in a set of crosses which did not contain the lethal in the  $bs^p$  chromosome. In  $F_1$   $px\ bl \times Px^2$ , extreme plexus and completely blistered flies  $bs^p/Px^2$  were obtained.  $F_2$  from these gave the results

shown in table 84. Considering the overlapping between the extreme and less extreme classes, and the ever-present shift toward the lower type in males, this represents the segregation into two  $Px_3/bs^p$  = extreme, one  $bs^p$   $px/bs^p$   $px$  = plexus  $\pm$  blistered and crossover  $bs^p$   $px/bs^p$ . The latter, web without  $px$  in females and a little lower type (antler web) in males, is the type of homozygous  $bs^p$ . The extracted types bred to expectation. The phenotype thus obtained assigns  $bs^p$  to a position between  $bs$  and Bridges's  $bs^1$ .

When stock  $bs^p$  was first received from Pasadena, it showed an extreme web formation where the antler is situated in  $bs$ , and a fine net formation in II. Later the type changed, showing a range of variability from EV up to the type just described. Blisters were never observed above the web. But later they again appeared in the stock and seem to be dependent upon the moisture conditions. The original phenotype, then, was a little higher than the type  $bs^p/bs^p$  isolated from  $px$   $bl$  stock. The compound  $bs/bs^p$  is not very different from a plus condition of  $bs$ , showing that no deficiency and no dominance are involved. The compound with  $px$   $bl$ , i.e.,  $bs^p/bs^p$ , is different from  $px$   $bs^p/bs$ . Besides the antler ( $\pm$ ) there are extra veins in I and II, the latter sometimes forming a net, and a branch to the cross vein. The effect of  $px$  in this case is not large, as the combination  $px$   $+/+$   $bs^p$  shows only the usual extra vein in I, a branch to the cross vein, and the heterozygous  $bs$  condition from  $+$  to EV. Most characteristic is the compound  $bs^p/Px^2$ . Here the usual exaggeration effect of the deficiency produces structureless, inflated, shortened and narrowed wings carried in a spread condition, a type which we call *sac*.

We have already stated that our standard  $bs$  stock contained in irregular distribution another  $bs$  allele which we call  $bs^{b1}$  and which is a still higher allele than  $bs^p$  of low viability and fertility. In reporting the  $px$   $bl \times bs$  crosses (see table 80) we mentioned that in some cases all  $F_1$  individuals showed the antler at CV, being the effect of the compound  $bs^p/bs$ . In other cases one-half of the  $F_1$  individuals were as described, the other half showed an exaggerated antler with web formation, a type of exaggerated  $bs$  action which we described for other compounds. The cross was afterward repeated many times. Sometimes a whole series never produced the exaggerated type; sometimes many or even the majority of crosses gave 50 per cent offspring of the exaggerated type. Never were all flies of this type. As the  $bs$  stock is usually responsible for the result, something is present there which fluctuates in numbers in the stock bottles and is either lethal or too unviable to appear in homozygous condition in the stock bottles. It turned out that the classification of  $F_2$  and backcrosses was very difficult on account of considerable overlapping in the presence of  $px$ , and therefore the exaggeration effect of the  $Px^2$  deficiency was again used for further tests.  $F_1$  web individuals were crossed to the Plexates, the nonweb  $F_1$  crossed to the same (already reported) serving as controls. This cross ( $px$   $bl \times bs$ ) web  $\times Px^2/Cy$  ought to yield, apart from the  $Cy$  half, one-half of the already analyzed  $px$   $bl/Px^2$  compound and one-half of the supposed higher  $bs$  allele ( $bs^{b1}$ ) opposite the deficiency. The latter would be recognized by the exaggeration effect, which has already been described for  $bs$ ,  $bs^p$ , and  $bs^2$ . Actually, one-half of the offspring from this cross showed the type *sac* just described for the  $bs^p/Px^2$  compound. The test was provided by either crossing many  $bs$  with  $Px^2$  or with  $(bs \times +) \times Px^2$ , in which case *sac* segregated whenever  $bs$  was heterozygous for  $bs^{b1}$ . Homozygous  $bs^{b1}$  was then to segregate from *sac* parents =  $bs^{b1}/Px^2 \times bs^{b1}/Px^2$  as one-third of the

offspring. An actual result from 17 such  $F_2$  was 836 females 797 males sac, 196 females 164 males  $bs^{b1}/bs^{b1}$ , showing the latter type rather unviable (about 50 per cent viability). Individual ratios are frequently very low, e.g., 16:1 instead of 2:1.

The segregating type  $bs^{b1}/bs^{b1}$  is very characteristic: it has a very extended web near the cross vein which reaches the edge of the wing. Above this web a blister is formed which is present in one or both wings. The type then closely resembles Bridges's  $bs^2$ , but is more extreme, a condition which is expressed in its low viability and considerable sterility. Many flies do not produce offspring, and those that do produce only few individuals. A stock could not be kept up for many generations, even with large numbers of parents used.

The stock  $bs^{b1}/Px^2 = sac$  derived from  $bs \times Px^2$  thus was phenotypically identical with one, also sac, derived from  $(px\ bl \times bs)$  type web  $\times Px^2$ . But it soon became clear that the two stocks were only phenotypically alike. In the stock not involving  $px\ bl$ , later tests always gave the expected results for  $sac = bs^{b1}/Px^2$ .  $Sac \times sac$  segregated  $bs^{b1}/bs^{b1}$  in less than one-third of the offspring, which bred true if at all. Further  $bs^{b1}/bs^{b1} \times Px^2$  gave only sac. We made the same tests with the sac derived from  $(px\ bl \times bs) \times Px^2$  by breeding from sac flies (in mass because of low fertility and viability), and again the offspring always segregated in sac and the high web type; the latter type crossed with  $Px^2$  produced only sac. But pairs of the high type resembling  $bs^{b1}/bs^{b1}$  only occasionally bred true; sometimes they segregated sac. We shall return to this strange result at once. Actually, this stock, which was always bred from numerous sac flies, changed after a long series of generations of typical behavior. The segregating homozygotes were now of a lower type, about like  $bs^2$ . These had now become perfectly fertile and they bred true. The new type was rather different from the former extracted homozygotes. The web did not reach the wing margin, but there was more or less of a parallel vein present; the wings were blistered, or not. Males were never blistered, though they were so in the extracted homozygotes. Crossed with the Plexates, they still produced sac. But, in addition, the sac type also had changed to something between extreme plexus blistered and sac, i.e., a little wing structure was left.

The clue to these incongruities was found when many of the  $bs^{b1}/bs^{b1}$  type flies derived from the cross involving  $px\ bl$  via sac were tested before these changes occurred. As has already been reported, many of these tests (checked over and over again for virginity because of the incongruous breeding behavior) produced both their own type and sac from both parents of the high web type, and were, besides, more fertile and viable. Among three of these segregating broods were a few  $bs^{b1}$  type flies with additional plexus, obviously crossovers, which required the presence of a  $px$  containing chromosome from  $px\ bl$  stock in the parents. A pair of such flies found in the stock bred true and was much more fertile than usual and also viable (2414). Obviously, this stock, which was supposed to be  $bs^{b1}/Px$  (sac) segregating  $bs^{b1}/bs^{b1}$ , contained an admixture of a compound of  $Px$  with another second chromosome from  $px\ bl$  carrying a different  $bs$  allele, namely what we previously called  $bs^{pp}$  present together with  $px$ , i.e.,  $bs^{pp}\ px/Px$ . We have already seen that  $bs^{pp}\ px$  homozygous is almost sac. As we had made up the stock by mass breeding on account of unviability of sac and  $bs^{b1}$ , by chance  $bs^{pp}$  had also entered the stock derived from  $(px\ bl \times bs/bs^{b1}) \times Px$ . Thus, among the phenotypes sac of this stock there were mostly  $bs^{b1}/Px$  and some  $bs^{pp}px/bs^{pp}px$  flies; the latter were a minority and



by chance this type was never used alone for breeding, which was always done with a few pairs of sac ( $bs^{pp}/Px^s$  is also sac). The phenotype  $bs^{b1}$  in the stock thus was either  $bs^{b1}/bs^{b1}$  or  $bs^{pp}px/bs^{b1}$ , and by crossing over  $bs^{pp}px/bs^{b1}px$  could also be obtained. A cross  $bs^{pp}/bs^{b1} \times bs^{b1}/bs^{b1}$  seemed to breed true; though the lower grade web of the  $bs^{pp}/bs^{b1}$  flies and their better breeding was noticed. A cross  $bs^{pp}/bs^{b1} \times bs^{pp}/bs^{b1}$  segregated the almost sac flies, which now bred true. Extracted F<sub>2</sub> agreed with these interpretations. Finally, the change which later occurred in the stock, as described, consisted first of a spreading by selection of the viable  $bs^{b1}/bs^{pp}$  type, which seemed to breed true, and the spreading of the  $bs^{pp}$  homozygous almost sac type. But there is a considerable selection against sac,  $bs^{pp}$  and  $bs^{b1}$ , as compared with the best viable  $bs^{b1}/bs^{pp}$ , which actually were predominant when last tested, whereas  $bs^{b1}/bs^{b1}$  (similar phenotype) had been very rare in the original stock.

Table 79, as already stated, contains an evaluation of the average phenotype of different combinations and compounds, among them a number not mentioned in the text. The description applies to nonisogenic stocks kept under more or less identical conditions. There is much variability and overlapping, as mentioned in the text, and there are modifiers, which have been traced, that increase or decrease the average extravenation. On the whole, however, the table gives a fair description of the types found in such actual experiments where a clear classification was possible.

It might finally be added that the degree of plexation produced by the  $px$   $bs$  combination in  $px$   $bl$  can be considerably enhanced by the presence of the Payne inversions in the third chromosome. This was noticed when test crosses were made with the dominant  $Sb$  (Stubble) associated with such an inversion, or not. The former produced exaggeration of  $px$ , the latter did not. The dominant  $S$  (Star) is known to act as a suppressor of plexation. The same was found for  $H$  (Hairless) in some crosses.

*Remarks on the phenogenetics of plexation.*—Though it does not actually belong to the problems of this paper, a short discussion of the phenogenetic features involved in the foregoing analysis of the plexus wing types may be added.

Looking over all the phenotypes recorded in these different heterozygotes and compounds, we realize that they all more or less obey a definite rule. Let us consider first the  $bs$  series, namely,  $bs$ ,  $bs^p$ ,  $bs^{b1}$ ,  $bs^a$ ,  $bs^{pp}$ , and their compounds, including the compounds with the Plexates. The lowest action of  $bs$  upon the wing is not visible, i.e., the wing is normal. The seriation of visible effects is perfectly orderly in the homozygotes, heterozygotes, and compounds, namely, a simple addition of the effects of each locus, within the range of a certain fluctuation. To this is added the perfectly orderly effect of the exaggerating deficiencies, orderly so far as the exaggeration parallels the series of the effects of the respective alleles opposite the deficiency. The order of the effects is: dot, dash, antler, large antler in V; broadening of the center of the antler into a web; large web, extreme web extending to wing margin, beginning blister above the web in one wing, both wings, considerable blistering, extreme blistered wing, almost sac and sac wing. Normally, the effect is then confined to the cell V, though occasionally other extra veins occur even in the absence of  $px/+$ . If the exaggerating deficiency is present, the action of this compound spreads to the other wing cells and the complete series of effects characterizing the  $px$  and  $ba$  action is found, again in their proper order of increase in the respective compounds.

The plexus (and ba) series is also an orderly one in homozygotes, heterozygotes, and compounds of px, and in exaggerated conditions produced by the simultaneous presence of mutation or deficiency at the bs locus, or modifiers of a different kind. The pattern for the px action is a different one from that for bs. It begins with extra veins in I, II, and IV, and only in the higher grades do the same elements appear as in the bs series in V. The seriation is, roughly: extra veins in I; in I and IV; in I, II, and IV;  $\pm$  periclinal vein,  $\pm$  parallel vein and antler and web formation, in II and V. In a general way, we may say that the action of bs and px is similar, but the first starts at the posterior part of the wing and proceeds there to a high grade before other parts are affected; px, however, starts at the anterior edge and in cell IV and spreads to the posterior part of the wing in the higher grades. If both mutant loci are present, they are additive; heterozygous loci, too, have an additive effect. The deficiencies at the bs and ba locus act upon both series of effects in a similar way.

It may be added that still other patterns exist. The mutant net (left end of second chromosome) shows a series strongly resembling the px series combined with the bs series. But here the antler formation at the fifth longitudinal vein is pushed still more toward the wing edge, where it extends toward the fourth vein and tends to supplant the periclinal vein by a broad mass of chitin near the wing edge, whereas the other extra veins do not increase considerably. There is an additional tendency toward net formation in the submarginal cell from the antler of the third vein.

One of the most important facts known for certain series of multiple alleles is that the genic effect takes place at different times in development, namely, progressively earlier the higher the alleles (details and literature in Goldschmidt, 1938). It would be interesting, therefore, to relate the series discussed here to developmental facts. A complete analysis of the development is not yet available. But a few facts are already known (see Waddington, 1940, and photographs in Goldschmidt, 1935a). In normal wing development in the pupa the third and fourth longitudinal veins are ahead in differentiation. The second vein is rather late, and the fifth lags far behind. The part of the fifth behind the cross vein is perfected latest of all. At the time when the third and fourth veins are clearly visible, the region of the fifth and of the cross vein is still a wide-open sinus. And even when most of the veins have reached their final shape the cross vein and the fifth are wide channels (see photographs in Goldschmidt, 1935a). Extra veins which become visible only late in development must be formed by incomplete concrescence of the wing membranes. If such a disturbance happens, its first indications ought to be where the regular veins are last perfected. This is the region of the third posterior cell (V) and of the cross vein. What little information is available thus far seems to indicate that in the bs series as well the low grades are produced by action late in development and the higher grades by earlier incidence of the disturbance.

We add only that blister formation is the consequence of very incomplete concrescence of the wing membranes, allowing the blood which is pressed into the wing at eclosion to enter between. The actual series of blistering agrees with the expectations on a time basis. At present this is as far as we can go. A detailed discussion might be useful after Waddington, who recently published a preliminary account of the development of venation, has presented the details. As the difference between the px and bs effects is mainly one of pattern, i.e., the start of the process at the

anterior and posterior edge of the wing, respectively, it might be pointed out that a similar difference exists for the vestigial and Beaded type of scalloping of the wing. In this case, Braun (1940) was able to show that the type can be shifted to one of the other pattern by changing the speed of larval development. It might be added, finally, that wing plexation can be produced in wild type by temperature action in a critical period as a so-called phenocopy (see Goldschmidt, 1935), just as is the case with other mutants already discussed. This shows that most interesting phenogenetic facts are here at hand which deserve further analysis, the more so when we add that the degree of plexation is enhanced by the same inversions which also enhance the degree of scalloping (see Gardner, 1942, and Goldschmidt and Gardner, 1942).

#### e. BLISTERING IN THE px bl STOCK

One of the characteristic features of the original px bl stock which remained constant for years in some of the stocks (without selection) was the regular incidence

TABLE 85  
BLISTERING IN px bl BROODS

No.	♀	♀ blist	♂	♀ both wings	♂ blist	♀ : ♀ blist
4019 B.....	35	39	50	..	..	0.9 : 1
4020 B.....	58	51	123	9	3	1.0 : 1
4021 B.....	38	49	105	2	..	0.7 : 1
4027 B.....	54	28	80	3	..	1.7 : 1
4028 B.....	63	45	104	..	2	1.4 : 1
4059 B.....	58	6	39	..	..	9.7 : 1
4060 B.....	62	16	87	..	1	3.6 : 1
4075 B.....	63	20	77	4	2	2.6 : 1
4077 B.....	68	22	98	..	..	3.1 : 1
4089 B.....	5	30	25	1	..	0.2 : 1
4090 B.....	63	29	95	3	..	2.0 : 1
4091 B.....	5	11	20	2	..	0.5 : 1
4096 B.....	27	19	70	2	..	1.3 : 1
4097 B.....	61	34	84	2	2	1.7 : 1
4099 B.....	24	30	45	4	..	0.7 : 1

of blisters, usually upon only one wing, in a high percentage of females and in only a few males. As the bs locus is involved, this blistering might be the phenotype of the bs homozygote or compound combined with homozygous px. The presence of blisters in only a part of the females and rarely in males would agree with the phenotypic effect found in some compounds with the Plexate deficiencies, as described above. The selection experiments within the stock (see below) might agree with such a simple interpretation, but the outcrossing experiments show it to be wrong.

*Blistering within the original px bl stock.*—One of the most conspicuous features of this line is the blistering of the wings. As a rule, only females are blistered and only one wing is blistered. When the incidence of blistering is high, females with both wings blistered, and blistered males, appear. The following experiments performed within the px bl line soon after it was found show that this blistering has a genetic basis and not a simple one. (The stocks have changed meanwhile; see below.)

In an experiment which involved breeding from different types of pairs over six

generations, females with one or both wings blistered and males with blisters were obtained (px and px blist describe only the phenotype, not the loci). The total numbers are given in table 85. This table shows that from all combinations a rather constant percentage of females with both wings blistered and blistered males were produced, namely, around 1.3–1.4 per cent. The double-blistered females and the blistered males appear in equal percentages and therefore are possibly due to the same genetic situation, actually a small overlap of incomplete penetrance.

TABLE 86

FEMALES WITH BOTH WINGS BLISTERED, AND BLISTERED MALES IN DIFFERENT px bl COMBINATIONS

Combination of parents	♀ px	♀ px blist	♂ px	♀ both wings blist	♂ blist	♀ both wings blist		♂ blist per cent of ♂	Per cent ♀ blist one wing
						Per cent of all ♀	Per cent of blist ♀		
♀ ♂ not blist...	3142	1181	4223	72	42	1.7	6.1	1.0	39.0
♀ blist ♂ not...	2703	1191	3577	48	50	1.3	4.0	1.4	44.0
♀ and ♂ blist...	1402	556	1778	20	34	1.0	3.4	1.9	39.6
♀ not ♂ blist...	337	129	489	4	7	0.9	3.1	1.4	38.3
	7584	3057	10067	144	133	1.4	4.7	1.3	40.3

Many tests were made to find out whether the percentage of blistered individuals has a genetic basis beyond the homozygous *bs*<sup>+</sup> locus together with *px/px*. External conditions may act to a certain extent in influencing the percentage, but the average production of blistered flies is independent of external conditions. Fifteen pairs of females and males without blisters gave offspring as listed in table 85.

This group shows a considerable diversity which might be more than fluctuation. The statistical tests show for an assumption of 40 per cent blistered as found in the grand total (table 86) a  $\chi^2=0.98$   $P>0.30$ ; but the homogeneity test is negative, i.e.,  $\chi^2=119.42$   $P<0.01$ . Thus the ratios are not significantly different from those found in table 86, but the series is probably not homogenous. From these broods the following selections were made:

- 1) Selecting from bottle 4059 with only 1/10 blistered flies, we got:

♀♂ not blistered, 4059:

No. 4222 13 ♀ 4 ♀ bl 13 ♂

No. 4223 104 ♀ 21 ♀ bl 173 ♂

which is a rather low ratio for blistered, indicating a genetic basis.

*F*<sub>2</sub> from not blistered, 4223, gave:

No. 4389 53 ♀ 12 ♀ blist. 67 ♂, which again is a low ratio.

*F*<sub>2</sub> from not blistered, 4389, gave:

No. 4539 38 ♀ 14 ♀ blistered 45 ♂

- 2) Selecting normals from bottles with a 1 : 1 ratio in the first generation, we found:

No. 4132 ♀♂ normal no. 4019: 80 ♀ 30 ♀ blist 104 ♂

No. 4133 ♀♂ normal no. 4019: 75 ♀ 91 ♀ blist 116 ♂

No. 4185 ♀♂ normal no. 4099: 94 ♀ 50 ♀ blist 126 ♂

which looks like the same ratios as were found before in the whole group.

*F*<sub>2</sub> from the last out of normals:

No. 4371 42 ♀ 16 ♀ blist 61 ♂, a rather low ratio

3) For normals selected from bottles with an intermediate ratio (namely, 4060) see table 87. All these selected  $F_2$  from an  $F_1$  showing a 3:1 ratio gave a very low ratio, namely about 8:1.

TABLE 87  
SELECTION IN px bl

No.	♀	♀ blist	♂
4224 B.....	98	9	117
4225 B.....	106	8	97
4226 B.....	101	13	105
4227 B.....	93	15	104
4228 B.....	76	10	85
Total . . . . .	474	55	508

In  $F_2$  and  $F_3$  of this set of selections, then, the same ratios reappeared as were found in the first generation, namely, high, medium, and low, and there was some effect of selection for low percentage of blisters.

TABLE 88  
SELECTION IN px bl

blist ♀ from no.	Ratio $F_1$ ca.	No. $F_2$	♀	♀ blist	♂	
4021	1:1	4124 B	51	31	92	Mother both wings blistered
4021	1:1	4125 B	51	48	102	
4021	1:1	4126 B	113	51	153	
4021	1:1	4127 B	80	26	126	
4021	1:1	4128 B	68	36	108	
4020	1:1	4129 B	40	17	70	
4020	1:1	4131 B	81	32	87	
4019	1:1	4134 B	89	33	120	
4019	1:1	4135 B	94	28	105	Low ratio
4019	1:1	4136 B	55	14	76	
4019	1:1	4137 B	111	25	145	
4019	1:1	4186 B	80	32	108	
4089	1:5	4288 B	9	4	21	
4089	1:5	4290 B	70	27	88	
4089	1:5	4291 B	87	38	127	
4089	1:5	4292 B	96	36	120	
4091	1:2	4312 B	20	50	61	Mother both wings blistered
4091	1:2	4313 B	33	55	70	Mother both wings blistered
4090	2:1	4315 B	44	77	91	Mother both wings blistered

A second set of crosses selected blistered females and their normal brothers from the same  $F_1$  (table 88). This shows that the blistered females with their normal brothers tend again to produce the same ratios as prevail generally. There are contained in this series four broods from parents with a large excess of blistered (1:5) which behave like the others. But three broods bred from females with both wings blistered produced an offspring with a 1:2 ratio. In a general way, then, the blis-

tered females breed like their nonblistered sisters, though the type with both wings blistered seems to have a genetic basis.

Only one F<sub>1</sub> from blistered females was bred:

No. 4445 (from ♀ blist. 4292): 89 ♀ 17 ♀ blist 90 ♂

There were two F<sub>2</sub> bred from normal sisters (out of blistered grandmothers) (see table 89). The results are of the type found before for nonblistered or blistered

TABLE 89

No.	Parent's no.	♀	♀ blist	♂
4335 B	4135	87	8	98
4336 B	4135	85	32	103

mothers. In F<sub>4</sub> from the F<sub>2</sub> 4335 with 1/11 blist. flies (from normal parents) were obtained:

No. 4513 65 ♀ 12 ♀ blist 59 ♂

No. 4537 42 ♀ 7 ♀ blist 5 ♂

i.e., again low ratios of blistered, apparently a successful selection.

A third group of selections from the same F<sub>1</sub> group was made with both parents blistered (see table 90). Again the same irregular results appeared and the number of blistered males was not increased. F<sub>2</sub> from these, again with both parents blistered, gave:

No. 4370 (from 4184) 95 ♀ 21 ♀ blist 119 ♂

No. 4442 (from 4269) 72 ♀ 20 ♀ blist 66 ♂

which is actually a lower ratio for blistered.

There is no need to go into the details of further generations. It turned out to be impossible to produce by selection either a line without blistered flies, or with only blistered females, or with males as well as females blistered, though all this was produced later after spontaneous changes had taken place in the stock. Only the low incidence of blisters seemed to yield to selection.

All this applies to work done with the stocks while they remained fairly constant. I have already mentioned that, more recently (1940), blistered flies disappeared from the stocks. As this happened in all the stocks, obviously some unknown feature (food?) must have made this type less viable so that the modifiers needed for blistering were counterselected. In this case, however, as opposed to the former experiments, selection was completely effective, as may be illustrated by one example:

1st generation (pair matings):

a) 54 ♀ 5 ♀ bl

b) 62 ♀ 2 ♀ bl

c) 17 ♀

2d generation from a blistered female:

91 ♀ 17 ♀ bl

3d generation from a blistered female:

74 ♀ 15 ♀ bl 2 ♂ bl among 90

4th generation:

a) from ♀ and ♂ bl:

49 ♀ 21 ♀ bl 76 ♂ 1 ♂ bl

b) from ♀ bl:

76 ♀ 46 ♀ bl 182 ♂ 3 ♂ bl.

Stock remained constant in following generations. But when checked a year later, this selected stock had again returned to little blistering.

We return to the selection experiments in the original stock, performed before 1936. As the sex ratio is about normal, both in the stock and in the selections, the absence of blistering in the males cannot be due to lethality. But we saw that the number of rare blistered males is the same as that of rare double-blistered females;

TABLE 90

P. no.	F <sub>1</sub> ratio	No. F <sub>2</sub>	♀	♀ blist	♂	♂ blist
4020	1 : 1	4183 B	31	13	43	..
4020	1 : 1	4184 B	36	14	45	2
4028	1 : 1	4192 B	49	17	68	..
4060	3 : 1	4269 B	87	28	100	1
4075	3 : 1	4296 B	16	37	47	..

further, that the males are always behind the females in plexation, and that in the just-reported broods with numerous blistered males their number is behind that of the females. We conclude, therefore, that whatever causes the blistering acts in the males only at a higher level than in the females. This conclusion will be borne out in crosses producing blisters by other means, as was shown to be true in the Px compounds.

In the following enumeration we tabulate the entire set of data, which include additional broods not mentioned in this chapter. Only the type of the parent of a brood is given, irrespective of the ancestors (blistered or not). All combinations were made within the original plexus blistered stock, i.e., before 1936.

1) 57 broods, both parents not blistered:

3152 ♀ 1181 ♀ blist. 72 ♀ both wings blist. 4223 ♂ 42 blist.

Sex ratio 1.03 : 1. Percentage ♀ blistered among ♀ = 28.4 per cent, ♂ bl = 1 per cent.

The ratio of n not blistered ♀ : 1 blistered ♀ is found in X broods:

n = 0-0.5	0.5-1.5	1.5-2.5	2.5-3.5	3.5-4.5	4.5-5.5	5.5-6.5	6.5-7.5 and above
X = 1	13	9	9	3	7	4	8

2) 26 broods, both parents blistered:

1402 ♀ 556 ♀ blist. 20 ♀ both wings blist. 1778 ♂ 34 ♂ blist.

Sex ratio 1.08 : 1. Percentage ♀ blistered among ♀ = 29.2 per cent, ♂ bl. almost 2 per cent.

The ratio of n not blistered ♀ : 1 blistered ♀ is found in X broods:

n = 0-0.5	0.5-1.5	1.5-2.5	2.5-3.5	3.5-4.5	4.5-5.5	5.5-6.5	6.5-7.5 and above
X = 2	2	5	5	5	1	1	1

3) 4 broods, ♀ not blistered × ♂ blistered:

337 ♀ 139 ♀ blist. 4 ♀ both wings blist.

489 ♂ 6 ♂ blist.

Sex ratio 0.97 : 1. Percentage ♀ blistered among ♀ = 30 per cent ♂ = 1.2 per cent.

Ratios ♀ : ♀ blist. : 1.2 2.4 3.9 4.4 : 1

4) 47 broods, ♀ blistered ♂ not blistered:

2703 ♀ 1191 ♀ blist. 48 ♀ both wings blist. 3577 ♂ 50 ♂ blist.

Sex ratio 1.09 : 1. Percentage ♀ blistered = 36 per cent ♂ = 1.4 per cent.

The ratios of n not blistered : 1 blistered ♀ are found in X broods:

n = 0-0.5	0.5-1.5	1.5-2.5	2.5-3.5	3.5-4.5	4.5-5.5	5.5-6.5	6.5-7.5 and above
X = 2	13	11	7	4	1	3	3 2

Conclusions : The foregoing data suggest that blistering in the stock, together with the high grade of plexation, is based upon a homozygous genetic condition, namely,

homozygosity of px bs with a series of special features: (1) There is a sex-limited expressivity, males tending to lower plexation and a threshold for appearance of blistering which is only rarely (1 per cent) surpassed. (But a higher allele of bs<sup>p</sup> has arisen repeatedly, which affected males almost as much as females.) (2) The

TABLE 91  
F<sub>2</sub> AND RF<sub>2</sub> OF px bl × N

Cross	No. of broods	Not px		px		blst	
		♀	♂	♀	♂	♀	♂
1. (px bl blist × N) <sup>2</sup> .....	12	740	536	279	178	25	1
2. (px bl × N) <sup>2</sup> .....	24	1446	1150	503	373	13	..
3. (N × px bl) <sup>2</sup> .....	16	1184	913	429	270	8	7
4. (N × px bl blist) <sup>2</sup> .....	7	572	469	149	114	..	..
5. (y × px bl) <sup>2</sup> .....	13	560	399	157	108	..	7
6. (px bl blist × N) × recipr.....	51	4940	4054	919	546	1	..
7. (N × px bl) × recipr.....	7	501	372	174	127	24	1
8. (px bl × N) × recipr.....	11	810	721	262	198	2	..
Total.....	141	10753	8614	2872	1914	63	16

percentage of blistering in females shows also an incomplete expressivity, as all-blistered lines cannot be selected. This may be a threshold condition, but it may also be due to special balances of modifiers. A threshold for penetrance is more probable. (3) There are modifiers present for lowering the expressivity of blistering which are subject to experimental as well as to chance selection under some environmental

TABLE 92  
DISTRIBUTION OF BROODS WITH BLISTERED FLIES AMONG THE EIGHT  
TYPES OF CROSSES FROM TABLE 91

F <sub>2</sub> broods without blisters.....	120
F <sub>2</sub> broods with blisters.....	29
of these latter in group 1.....	8 out of 14
2.....	7 out of 25
3.....	2 out of 19
4.....	2 out of 9
5.....	3 out of 13. Only ♂
6.....	1 out of 51. (Only one ♀)
7.....	5 out of 7. Mostly ♀
8.....	1 out of 11. Only ♀

conditions, and for which return selection is possible. But they never could be selected beyond an average expressivity of 3 not blistered : 2 blistered.

*Blistering after outcrossing.*—If blistering were only the fluctuating effect of the homozygous px bl second chromosomes, F<sub>2</sub> from crosses ought not to differ from px bl within the plexus flies containing both px bl second chromosomes except for crossing over of eventual second-chromosome modifiers. Actually this is not the case. Out of about 150 such F<sub>2</sub> crosses about four-fifths of the broods with thousands of flies did not contain a single blistered fly though the mother or father came from



a px bl line with high incidence of blisters or were themselves blistered. Tables 97-93 contain the details. Table 91 lists the types of crosses in eight different categories. (px bl  $\times$  N)<sup>2</sup> means F<sub>2</sub> from px bl females with a male of some standard stock not containing dominants, deficiencies, known rearrangements, or px or bs or ba alleles; px bl blist means that the P ♀ or ♂ was blistered. The mark  $\times$  recipr. means the double reciprocal cross, which introduces different X chromosomes into

TABLE 93  
SEGREGATION IN CROSSES PRODUCING BLISTERED FLIES, AS SUMMARIZED IN TABLE 92

No.	Cross type	not px		px		px blist		ratio not px : 1 px		
		♀	♂	♀	♂	♀	♂	♀	♂	
73	1	42	27	33	17	1	..	1.4	2.0	2 ♀ px bl w (w $\times$ px bl) <sup>2</sup> 1 ♀ px bl w (px bl $\times$ w) <sup>2</sup> (px bl $\times$ w) <sup>2</sup> (px bl $\times$ w) <sup>2</sup>
74	1	68	50	22	14	1	..	3.1	3.6	
76	3	40	47	35	25	3	3	1.1	1.4	
78	3	76	73	35	15	6	4	1.8	3.8	
84	1	79	61	44	30	4	..	1.6	2.0	
85	1	85	12	29	9	7	..	2.4	3.2	
86	1	73	63	13	19	8	..	3.5	3.3	
396	1	50	41	24	18	1	1	2.4	2.3	
406	2	47	53	23	18	2	..	2.1	3.1	
416	7	58	40	20	7	4	1	3.1	6.1	
417	7	72	65	25	26	5	..	3.1	2.5	Count not reliable
418	7	71	50	28	19	3	..	2.7	2.6	
448	8	62	62	27	21	2	..	2.4	3.0	
452	7	53	50	22	10	4	..	2.6	5.0	
453	7	51	54	28	19	8	..	2.1	2.8	
463	4	47	32	17	..	2	1	2.5	32	
473	2	76	54	20	5	1	..	4.	11	
475	2	44	30	19	5	1	..	2.2	6	
476	2	41	25	24	19	3	..	1.8	1.3	
482	2	61	55	14	13	1	..	4.4	4.2	
483	2	61	48	24	13	4	..	2.7	3.7	
485	1	75	52	23	3	2	..	2	17.3	
487	1	71	48	25	10	1	..	2.7	4.8	
254	5	38	26	11	9	..	3	3.5	2.2	
261	5	31	19	11	4	..	2	2.8	3.1	
290	5	52	44	18	11	..	2	2.9	3.4	
456	2	57	44	26	16	1	..	2.1	2.8	

Further, one type 4 not properly classified.

the F<sub>2</sub> females as compared with the simple F<sub>2</sub>. The broods with and without blistered flies are listed in table 92. (In tables 92 ff. a few broods have been added not contained in table 91 because only the px flies had been counted.) There is only one group, 6, almost completely without blistered flies, that is, one brood out of 51 with one blistered female. In three groups, 1, 2, 7, many broods contain blistered flies; in the groups 3, 4, 8 only few broods contain blistered flies, and group 5 contains only blistered males. The groups 1, 2, 7 contain crosses which all produce in F<sub>2</sub> one-half females with both X chromosomes from px bl stock. In the groups 3, 4, 6, 8 such a combination is not possible. In group 5, the y cross, only males receive an X chromosome from px bl. This grouping suggests that the presence of the two X

chromosomes (or one hemizygous X) from px bl stock allows for or increases the production of blistered flies, whereas the heterozygous condition of the px bl X only rarely allows the appearance of blistered flies. In some way, then, the X chromosome is involved.

Tables 91 and 92 demonstrate that, in the groups of  $F_2$  which permit a recombination of two X chromosomes from px bl (1, 2, 7), 20 out of 46 broods produce blistered flies. In the groups which do not permit  $F_2$  combinations of two X chromosomes from px bl, namely, 3, 4, 5, 6, 8, only 6 out of 103 broods contained blistered females, altogether about a dozen individuals among thousands of flies. Among these are the double-yellow crosses (5) without px bl X chromosomes ( $\varnothing$ ) and without blistered females, and the huge series 6 with 51 broods and only one blistered fly. This suggests that typically no blistered females are found in the absence of the two X chromosomes from px bl stock, but that a few exceptions exist which have to be accounted for. They may be modifications or results of a rare crossing over.

The details of the  $F_2$  broods containing blistered flies are found in table 93. We begin with group 7, where 5 out of 7 broods contained blistered flies. All 5 belonged to one set of experiments (see below, table 94) in which all combinations of type 7 threw blistered flies. Type 7 is the double reciprocal cross  $(N \times px\ bl) \times (px\ bl \times N)$ . It differs from all other combinations leading to two X from px bl in  $F_2$  in so far as the  $F_1$  mother received her X from the px bl father. We might therefore assume that the enhancer for blistering is always (or almost always) present in the px bl males. We remember from the analysis of pointed that the male X chromosome is different from the female ones, by always containing a transposition because one class of crossovers between the points involved is lethal. The ratio of females px : px blist in the  $F_2$  crosses, type 7, is 123 : 24. If we assume on the basis of the former discussion that only females with both X from px bl are blistered, the ratio of blistered among these females is  $\frac{147}{2}$  — 24 : 24, i.e., 50 : 24 or 2 : 1, i.e., the ratio frequently found in the px bl line (see below).

In types 1 and 2 both X chromosomes from px bl recombined in  $F_2$  are derived from the original mother. If a homozygous enhancer is needed for blistering, the situation does not differ from that in class 7. The normal broods which appear, more than one-half of the broods, suggest another complication, which will be discussed later. This group of data is, then, in favor of the need for homozygosity of the px bl X chromosome for the enhancing of blistering. The ratio of blistered flies in groups 1 and 2, calculated as before, is  $\frac{401}{2}$  — 38 : 38 = 162 : 38 = about 4 : 1. This is a lower ratio than before. Looking at the data, it seems to be the average of two different groups: one with a high percentage, namely, 34 : 22, and another with a low percentage, namely, 129 : 16, both found in px bl stock, and indicating some other modifying influence, not yet accounted for.

We turn now to the large group of  $F_2$  which does not contain two px bl X chromosomes, where we found 97 out of 103 broods without blistered flies. Of the six broods with blistered flies, one belongs to type 8, with 2 blistered females among 29 px females, (i.e., in the group, 2 among 264); one belongs to type 6, with 1 blistered female (i.e., 1 among 920); and two belong to type 4. One of the latter is not included in table 93 because the flies were checked only for highest-grade px. In one

(yvf  $\times$  px bl blist)<sup>2</sup>, no. 463, there was 1 female each in the + and y (crossover) class blistered. In the other (464) there were 3 ♀ +, 1 y, and 1 vf blistered, which among 68 ♀ (+ and px) is a rather high percentage. No interpretation for these exceptions

TABLE 94

SERIES OF F<sub>2</sub> CROSSES MADE SIMULTANEOUSLY BETWEEN px bl AND y v f; RELATED GRANDPARENTS AND PARENTS

No.	Type	F <sub>1</sub> no.	not px		px		not y v f blist	
			♀	♂	♀	♂	♀	♂
396	1	273	57	41	23	18	1	..
406	2	275	49	53	21	18	2	..
407	2	276	69	70	32	30	..	..
458	2	322	39	36	20	14	..	..
461	2	323	73	47	19	21	..	..
472	2	351	89	57	30	11	..	..
473	2	351	77	54	19	5	1	..
474	2	351	51	47	19	17	..	..
475	2	346	44	30	19	15	1	..
476	2	346	44	25	21	19	3	..
477	2	346	53	26	15	12	..	..
478	2	346	35	35	15	10	..	..
479	2	346	41	40	9	12	..	..
480	2	346	39	31	11	11	..	..
481	2	346	55	51	16	13	..	..
482	2	337	62	55	13	13	1	..
483	2	337	65	48	20	13	4	..
499	2	367	45	14	12	4	..	..
500	2	367	47	56	42	21	..	..
502	2	367	59	49	29	13	..	..
390	4	267	61	55	24	29	..	..
391	4	267	47	50	21	21	..	..
494	3	360	51	68	20	20	..	..
495	3	360	96	75	27	24	..	..
496	3	360	55	43	12	13	..	..
497	3	360	86	77	37	13	..	..
414	6	272 $\times$ 267	44	22	13	5	..	..
433	6	274 $\times$ 269	61	16	16	17	..	..
416	7	270 $\times$ 275	62	41	16	6	4	1
417	7	270 $\times$ 275	77	65	20	26	5	..
418	7	270 $\times$ 275	74	50	25	19	3	..
452	7	309 $\times$ 305	57	50	18	10	4	..
453	7	309 $\times$ 305	59	54	20	19	8	..
444	8	295 $\times$ 294	62	73	20	17	..	..
445	8	295 $\times$ 294	67	59	18	18	..	..
448	8	295 $\times$ 294	64	62	25	21	2	..
492	8	367 $\times$ 361	97	69	26	17	..	..
493	8	363 $\times$ 361	91	64	25	27	..	..

can be offered aside from the always available statement that some unknown modifier was introduced into the cross. (Also a rare dominance effect of the X-chromosome condition might be invoked, or a multiple crossover.) The two remaining exceptions, nos. 76 and 78, table 93, belonging to crossing type 3, are remarkable.

Here a relatively high percentage of blistering occurred; further blistered flies were found in + and w ♀, i.e., heterozygous for the px bl X chromosome and those without it, but for undetectable crossovers—cross: (m × px bl)<sup>2</sup>; finally, there were about as many blistered males as females, a very unusual feature (both F<sub>2</sub> were bred from brothers and sisters). We shall soon describe a similar case in which another bs allele (bs<sup>pr</sup>) was responsible for the result. As no further analysis was made at the time, we can only register the exceptional case, though it is almost certain that it was another instance of crosses involving bs<sup>pr</sup> (see below).

In order to demonstrate the regularities which have been observed thus far, we present in table 94 a series of 38 F<sub>2</sub>, all made in different directions (see column "type") between the same px bl line with high incidence of blisters and a y v f stock. All crosses were made within three days and kept side by side. Frequently a series of F<sub>2</sub> were bred from brothers and sisters (see column F<sub>1</sub>). We see that among 38 broods 25 had no blistered flies; among 19 broods of type 2, 6 had blistered flies; the only brood in type 1, the same; and all 5 broods of type 7 contained blistered flies. These are the three types with both px bl X chromosomes present in half the F<sub>2</sub> females. Groups 3 and 6 had no blistered flies in 6 cases; and groups 4 and 8 together had 1 case among 8 (to which the two exceptional cases 463 and 464 just discussed ought to be added). We note especially that the crosses in type 7, all of which gave a high percentage of blistered flies, were derived from 4 different F<sub>1</sub>. The rules found for the whole series hold clearly for this strictly comparable series and are not chance products of environmental or genetic modification.

We have finally to report upon the behavior of F<sub>3</sub> derived from F<sub>2</sub> with and without blisters, bred, of course, from px flies. F<sub>2</sub> without blisters, independent of the type of the cross (except the y crosses), may produce all imaginable types in F<sub>3</sub>. For example, 8 F<sub>3</sub> from F<sub>2</sub> type 3 without blisters produced 2 broods without blisters, a condition which remained in further generations; further, there were produced 4 broods containing a small percentage of blistered flies (about 5 per cent), and 2 broods with about one-half blistered flies, which latter continued breeding like ordinary px bl stock. Similar results were obtained from type 2 F<sub>2</sub>. For example, from the F<sub>2</sub> type 2 tabulated in table 94, F<sub>3</sub> was bred without and with blistered flies. The former bred true to nonblistering, the latter produced the different types, among them also the typical px bl condition with  $\pm \frac{1}{3}$  blistered flies. Example: F<sub>3</sub> from 482: (no. 558) 53 ♀ px 36 ♀ blist, 56 ♂ px 2 ♂ blist. Finally, marked third and fourth chromosomes recombine freely with blistering, so that the third and fourth chromosomes are hardly involved. It ought to be added here that neither svr<sup>po</sup> nor bran are present, as one might suspect in view of the fact that bran + poi = soft blistered. But in this case all ♀♀ and ♂♂ are blistered and, in the presence of px, are hardly viable (see above). In addition, no bran or poi ever segregated in these crosses.

We must now try to understand the facts and to relate them to the normal condition in the px bl stock. The following facts have to be brought into line.

- 1) In the original px bl stock percentages of 20–50 per cent blistered females and only 1 per cent males were constant.

- 2) Blistered and nonblistered ♀♀ and ♂♂ produced the same offspring.

- 3) Selection for absence or higher incidence of blisters was practically ineffective, though some result was obtained with low incidence of blisters.

4) The *bs* allele *bs*<sup>p</sup> in *px bl* does not produce blisters, either alone or in combination with *px*, but considerably so in compound with a *bs* deficiency.

5) Sometimes there is present in *px bl* a higher allele *bs*<sup>pp</sup> which in compound with *bs*<sup>p</sup> produces blisters in both sexes in the presence of *px/px*. It seems always to be linked with a lethal.

TABLE 95

VARIATION OF THE F<sub>1</sub> PHENOTYPE IN DIFFERENT *px bl* CROSSES, NAMELY, + × *px bl* (TABLE 95), *px bl* × + (TABLE 96) AND  $\bar{y}$  × *px bl* (TABLE 97)

No.	Cross	♀				♂			
		CV + EV	CV	EV	+	CV + EV	CV	EV	+
1	♀ + × ♂ <i>px bl</i> .....	23	6	20	..	..	..	30	11
2	♀ + × ♂ <i>px bl</i> .....	55	30	8	8	9	21	12	52
3	♀ + × ♂ <i>px bl</i> .....	44	20	9	10	1	5	13	54
4	♀ + × ♂ <i>px bl</i> .....	39	45	6	9	6	12	9	54
5	♀ + × ♂ <i>px bl</i> .....	28	55	6	25	3	9	13	87
6	♀ + × ♂ <i>px bl</i> .....	38	5	11	4	..	1	25	24
7	♀ + × ♂ <i>px bl</i> .....	10	7	..	1	..	2	1	4
8	♀ + × ♂ <i>px bl</i> .....	29	17	9	7	1	3	2	15
9	♀ + × ♂ <i>px bl</i> .....	45	9	7	8	..	6	8	44
10	♀ + × ♂ <i>px bl</i> .....	15	19	2	5	..	7	4	16
11	♀ + × ♂ <i>px bl</i> .....	20	27	7	27	1	3	5	81
12	♀ + × ♂ <i>px bl</i> .....	29	25	3	6	1	1	9	32
13	♀ + × ♂ <i>px bl</i> .....	26	20	5	5	1	4	11	27
14	♀ + × ♂ <i>px bl</i> .....	34	20	5	3	..	8	6	71
15	♀ + × ♂ <i>px bl</i> .....	17	23	2	6	..	4	4	52
16	♀ + × ♂ <i>px bl</i> .....	37	15	6	1	3	6	12	25
17	♀ + × ♂ <i>px bl</i> .....	37	25	3	2	8	7	4	40
18	♀ + × ♂ <i>px bl</i> .....	20	65	1	13	2	17	1	61
19	♀ + × ♂ <i>px bl</i> .....	23	53	14	36	4	14	12	82
20	♀ + × ♂ <i>px bl</i> .....	20	47	7	30	4	7	12	76
Total.....		586	531	131	206	44	147	193	888

6) Outercrosses show that (in the absence of *bs*<sup>pp</sup>) the condition of blistering found in *px bl* requires the presence of the original intact X chromosomes (in the presence of the original second chromosome).

7) The crosses with *px bl* ♂ suggest that the enhancer might be a transposition identical with or comparable to the one described for the X chromosome of pointed.

But this still cannot be the entire story (aside from modifiers). If the blistering is only produced by the combination of the original first and second chromosomes, all F<sub>2</sub> flies from broods which segregate this combination in one-half of the *px* flies ought to behave like the pure *px bl* stock except for crossing over. But actually half of the broods do not contain blistered flies, and it is possible to breed from such F<sub>2</sub> constantly normal as well as constantly blistered lines. It must therefore be assumed that in *px bl* a condition exists which is not necessarily present in extracted F<sub>2</sub> homozygous for both second and first chromosomes. As such a condition cannot be referred to enhancers in other chromosomes, the assumption is forced upon us that

some balanced condition exists within the px bl stock which prevents the formation of the types of broods without blisters, which, however, can be obtained after crossing.

We anticipate here that in the right end of the second chromosome of px bl two small deficiencies are found in the salivaries, both heterozygous. The first is probable always present (i.e., in half of the slides px bl  $\times$  +), namely, a one-band deficiency (?) at the px locus (see discussion below); the second, a two-band deficiency

TABLE 96

No.	Cross	♀				♂			
		CV + EV	CV	EV	+	CV	CV + EV	EV	+
1	♀ px bl $\times$ ♂ +	42	25	11	7	7	1	1	27
2	♀ px bl $\times$ ♂ +	32	12	2	1	4	..	1	16
3	♀ px bl $\times$ ♂ +	20	37	2	8	12	2	2	30
4	♀ px bl $\times$ ♂ +	34	24	2	2	18	..	3	50
5	♀ px bl $\times$ ♂ +	21	50	1	2	14	..	1	28
6	♀ px bl $\times$ ♂ +	45	10	1	..	14	5	2	24
7	♀ px bl $\times$ ♂ +	22	44	2	6	18	..	2	23
8	♀ px bl $\times$ ♂ +	25	31	1	3	8	..	..	25
9	♀ px bl $\times$ ♂ +	30	9	4	4	12	1	4	34
10	♀ px bl $\times$ ♂ +	21	21	..	3	26	4	..	27
11	♀ px bl $\times$ ♂ +	30	17	6	1	18	3	2	30
12	♀ px bl $\times$ ♂ +	63	23	2	1	36	11	5	32
13	♀ px bl $\times$ ♂ +	15	15	1	2	4	3	1	10
14	♀ px bl $\times$ ♂ +	31	46	..	2	14	1	..	24
15	♀ px bl $\times$ ♂ +	23	36	9	12	8	4	1	39
16	♀ px bl $\times$ ♂ +	25	6	5	2	14	4	2	21
17	♀ px bl $\times$ ♂ +	22	6	3	1	3	4	4	18
18	♀ px bl $\times$ ♂ +	..	4	1	..	7	..	1	8
19	♀ px bl $\times$ ♂ +	13	32	2	20	6	1	..	34
20	♀ px bl $\times$ ♂ +	25	38	2	5	20	2	3	41
Total		539	486	57	82	264	46	35	541

to the right of bs ba, is less frequent. Together they could provide a balance for the second chromosome. We know already that px bs<sup>p</sup>/px bs<sup>p</sup> has no blisters in the presence of the proper XX. If we call the px deficiency px<sup>p</sup>, the combination px<sup>p</sup>bs<sup>p</sup>/px bs<sup>p</sup> is also without blisters or with only rare blistered flies in the absence of the proper X chromosomes. But in the presence of these X chromosomes we find the standard incidence of blisters. Thus a cross px<sup>p</sup>bs<sup>p</sup>/px bs<sup>p</sup>  $\times$  + gives two types of F<sub>1</sub>, namely, px<sup>p</sup>bs<sup>p</sup>/+ and px bs<sup>p</sup>/+. F<sub>2</sub> from the latter will not produce blistered flies even with the original two X chromosomes. This, then, would explain the actual results. A check could be derived from the ratios of segregating plexus. In the presence of px<sup>p</sup>bs<sup>p</sup>/+ F<sub>2</sub> could be obtained from both parents of this constitution, which means that only a few px flies (from crossover px-bs) could appear if px<sup>p</sup>/px<sup>p</sup> is lethal. The data do not answer this question.

Another test was tried, namely, a careful phenotypical check of F<sub>1</sub> and the breeding of F<sub>2</sub> from different types. Actually, F<sub>1</sub> seemed to indicate the presence of a

heterozygotic condition in the *px bl* parents. Twenty  $F_1$  crosses each were made *px bl*  $\times$  Oregon and reciprocal with the then constant *px bl* stock, throwing about 20–40 per cent blistered females. In addition, the cross *y*  $\times$  *px bl* was made as a check for the X-chromosomal effect.

In tables 95–97 the results of these three groups of 20  $F_1$  each are tabulated. CV means individuals with branch at the cross vein, EV = extra vein in 5th cell (V), CV + EV = both, + = none. We notice that in both reciprocal crosses about  $\frac{1}{2}$  of the

TABLE 97

No.	Cross	♀				♂			
		CV + EV	CV	EV	+	CV + EV	CV	EV	+
1	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	12	14	..	..	..	5
2	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	14	37	..	..	..	51
3	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	..	20	..	..	..	19
4	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	8	20	..	..	..	21
5	♀ <i>y</i> ♂ <i>px bl</i> .....	..	1	24	28	..	..	10	56
6	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	19	20	..	..	8	29
7	♀ <i>y</i> ♂ <i>px bl</i> .....	1	..	13	20	..	..	1	27
8	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	13	19	..	..	2	47
9	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	24	17	..	..	5	33
10	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	18	13	..	..	9	39
11	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	11	12	..	..	6	50
12	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	14	29	..	..	..	54
13	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	26	5	..	..	3	14
14	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	23	32	..	..	6	51
15	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	13	17	..	..	5	36
16	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	20	19	..	..	7	35
17	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	6	23	..	..	..	39
18	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	14	21	..	..	2	98
19	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	15	24	..	..	..	46
20	♀ <i>y</i> ♂ <i>px bl</i> .....	..	..	15	21	..	..	1	45
	Total.....	1	1	302	412	..	..	65	746

♀ have the extra vein, whereas the majority possesses the branch of the cross vein. In most cases only a few + individuals are found, which might represent minus individuals of the CV class. But in a few cases large numbers of plus males seem to be significant. In the males, however, the plus individuals are in the majority, and only a minority of CV and EV are found. But we have to be prepared here, as always in these crosses, for a shift to the minus side in the males. Most conspicuous is, however, the difference in the *y* crosses. The second chromosome is heterozygous as in the others, but in the females the X from *px bl* are absent. In these females only two individuals with a branch of the cross vein were found, and in the males the CV class is completely absent. EV transgressing into + is known to be the heterozygous *bs* effect, with few + flies among the females and many among the males. CV is then supposed to be a heterozygous *px* effect. In the females there is certainly no segregation for the CV effect. In the males it looks as if such a segregation were visible in *px bl*  $\times$  plus, but not clearly in the reciprocal cross. Thus if the mothers

were actually  $px^{bs}/px^{bs}$  in  $F_1$  ♀  $px^p/+$  and  $px/-$  are not distinguishable, though possibly this is the case in males. But in the males there is apparent also a considerable shift toward normal in the absence of the  $px^{bl}$  X chromosome. Most disconcerting are the perfectly regular results of the  $y$  crosses with practical absence of the CV classes. As the females do not have any X chromosome from  $px^{bl}$  stock, one might think that this is responsible. But the males have such an X. We remember, however, that in pointed the male X differs from the female by always containing the transposition. Whether the same condition is involved here or whether  $y$  introduced a dominant modifier could not be decided.

Thus it was hoped that blistering and  $px$  segregation in  $F_2$  from selected  $F_1$  could furnish more information. Many  $F_2$  were bred from the different types of  $F_1$  and checked for all classes. Blistering occurred according to the rules discussed above, and  $px$  segregated in the different ratios to be discussed, but no clear correlation could be found. Thus it could not be decided whether the peculiarities of the inheritance of blistering were based upon  $px^p$ , or an independent dominant modifier, or the presence of another  $bs$  allele otherwise indistinguishable in its effects. The data to be reported below on the allele  $bs^{pp}$  make me favor the explanation by the  $px$  deficiency. It ought to be added that the localization of the X-chromosome enhancer for blistering did not succeed because among the few blistered  $F_2$  flies in crosses with marked X only very few were found in crossover classes, as has already been noted.

A short general remark may be added regarding blistering apart from the blistering involving the  $bs$  and  $ba$  stock. Blisters have been described also in translocation and similar stocks, though I do not know of a special inquiry into their appearance. In practically all stocks an occasional fly with blisters is found as a not heritable condition, as it seems, and in temperature experiments blistered wings may be produced as a phenocopy. I noticed years ago that a wild stock from Lausanne, kept in my Berlin-Dahlem laboratory, regularly threw blistered flies. Twenty-four pair matings in three generations yielded 3300 ♀♂, 30 ♀ 3 ♂ blistered. Fifteen of the twenty-four broods contained 1-5 blistered flies. Among the offspring of blistered flies the same ratio appeared, namely, 950 ♀♂, 10 ♀ blistered. Other wild stocks bred side by side with the Lausanne stock hardly ever contained a blistered fly. There was certainly some genetic reason for the behavior of the Lausanne stock.

*Blistering due to the allele  $bs^{pp}$ .*—At the time when we were trying to analyze the complicated situation regarding the blistering in the  $px^{bl}$  stock at the same time that the selection experiments were performed (see p. 423) an unexpected result was realized. From a pair of normal  $px^{bl}$  flies of the usual type and from a typical line throwing 30-50 per cent blistered females, a brood was derived:

4059 58 ♀  $px^{bl}$  6 ♀ blist 39 ♂

i.e., an excessively low percentage of blistered females for this line, and a low sex ratio.

From this, 2 broods from not blistered pairs were derived:

4222 13 ♀ 3 ♀ blist 13 ♂

4223 104 ♀ 19 ♀ blist 2 ♀ both wings blist 173 ♂

again a low percentage of blistered females, no blistered males. In the next generation 2 broods from normal females with brothers were bred:

4389 53 ♀ 12 ♀ blist 65 ♂ 2 ♂ blist

4390 1 ♀ 1 ♀ blist 5 ♂



From 4389 the following generation was bred :

a) From not blistered parents:

4539 38 ♀ 14 ♀ blist 44 ♂ 1 ♂ blist

b) From both parents blistered:

4540 35 ♀ 35 ♀ blist 3 ♀ both wings blist 73 ♂ 7 ♂ blist  
(3 ♂ hemithorax)

From both blistered parents, then, a high percentage of blistered females (50 per cent as opposed to 11 per cent in the first generation) was produced; further, a relatively high number of females with both wings blistered; and, finally, a very high percentage of blistered males (10 per cent; normally, 1.5 per cent). Again, a generation from both parents blistered was bred.

4677 23 ♀ 48 ♀ blist 70 ♂ 5 ♂ blist, i.e., more blistered females than not, and comparatively many blistered males

The next generation, 4856, had the same appearance but was not counted. But from this a stock was derived which contained almost exclusively blistered females and a high percentage of blistered males. Obviously the blistered male, father of 4540, had started the new condition, which bred true. It could be explained if there had arisen by mutation (or been rarely present in the stock) a higher allele of *bs*, namely, *bs*<sup>sp</sup>, which in heterozygous condition increased the percentage of blistering in both sexes. At that time it was suspected that this aberrant behavior might be connected with a deficiency in the second chromosome. Therefore, the different types found in the stock (♀♂ not blistered and blistered) were crossed to *b pr vg a sp* (5 ple). No deficiency for these loci was present. Simultaneously, another line derived from *px bl* in which blistering had completely disappeared (*px bl ll*) was tested the same way and no deficiency was present. Now the two lines, *px bl ll* without blisters and low *px*, and *px bl* 4856 with extreme incidence of blisters (and plexation), were crossed. The result was:

5015 *px bl ll* × 4856 ♂ not blistered:

7 ♀ *px* 31 ♀ *px* blist, about half both wings

38 ♂ *px* 4 ♂ *px* blist

5017 4856 ♀ blist × *px bl ll*

3 ♀ *px* 14 ♀ blist, mostly both wings

37 ♂ 2 ♂ blist

This cross, in both directions, then increased the blistered females to more than 80 per cent and simultaneously increased the amount of blistering (both wings). Two *F*<sub>2</sub> were bred, one from 5017 with normal flies (5236), one from 5015 with blistered flies (5240):

5236 = 5017<sup>♀</sup> not blist:

12 ♀ 18 ♂ not blist

19 ♀ 8 ♂ blist

5240 = 5015<sup>♀</sup> blist:

42 ♀ 109 ♂ not blist

47 ♀ 42 ♂ one wing blist

52 ♀ 28 ♂ both wings blist

In the larger brood, about  $\frac{2}{3}$  of the females and nearly  $\frac{1}{2}$  of the males were blistered, and about  $\frac{1}{2}$  of them on both wings. The new condition of high blistering was thus  $\pm$  dominant in both sexes in the presence of homozygous *px* and of the *bs* allele *bs*<sup>sp</sup>, and the 2:1 ratio for females indicates lethality of the homozygote.

The 4856 stock was now tested in numerous crosses with the loci at the end of the second chromosome (px, bs, ba, a, sp, ll). Not a single case of deficiency was found, the only distinction from typical  $F_1$  being that among thousands of flies 3 ♀ and 2 ♂ were blistered (i.e., a little dominance in the heterozygote). It ought to be mentioned that tests for the presence of pointed or bran were negative.

The next test was to find whether, as in ordinary blistering, the X chromosome was involved by combining the new condition with marked X chromosomes. It turned out that this blistering combined freely with foreign X chromosomes and

TABLE 98

TWELVE BACKCROSSES OF  $F_1$  plexus blistered STOCK × white OR vermillion (6 EACH), WITH plexus blistered white (OR vermillion)

(Formulae in text. The first six are the white, the others the vermillion crosses.)

No.	+		w (v)		px		px w (v)		px bl		px bl w (v)		+ bl web		(v) w bl web	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
8369 B.....	21	21	10	25	2	20	3	19	12	3	5	4	..	2	..	..
8370 B. ....	12	40	8	41	3	23	2	33	3	11	12	9	..	..	..	..
8371 B.....	17	22	15	23	5	14	5	14	8	7	12	4	1	..	..	..
8372 B.....	24	30	16	24	7	19	7	20	18	6	8	5	..	..	..	..
8374 B.....	18	40	9	34	3	27	..	24	10	23	12	8	..	..	..	..
8375 B. ....	14	27	18	13	6	19	10	21	2	1	2	..	..	..	..	..
8377 B. ....	22	28	13	26	6	16	7	20	24	2	13	6	..	..	..	..
8378 B.....	43	37	56	20	29	34	29	34	11	..	23	1	..	..	..	..
8379 B.....	16	25	27	12	11	22	3	15	17	7	8	7	..	..	1	..
8380 B.....	26	17	16	19	11	15	4	14	10	2	11	5	..	..	1	2
8381 B. ....	21	24	19	20	4	23	7	11	14	3	15	5	..	..	3	3
8383 B.....	25	21	20	13	6	16	6	14	14	4	14	9	..	..	..	2
Total w.....	106	180	76	160	26	122	27	131	53	51	51	30	1	2	..	..
Total v.....	153	152	151	120	67	126	56	108	90	18	84	33	..	..	5	7

was completely (or almost) autosomal in origin. This permitted a test of the blistering effect in homozygous and heterozygous condition. Blistered females from the blistered stock were crossed with white (w) males and the  $F_1$  females backcrossed to formerly extracted male px bl, w and blistered. If the blistered stock was homozygous  $bs^{pp}$ , the cross was:

1)  $px\ bs^{pp}/+, w/+ \times px\ zs^{pp}/px\ bs^{pp}, w$ . The expectation is  $\frac{1}{2}$  like stock (with or without w),  $\frac{1}{2}$  w or +. This means that almost all px females and fewer males are blistered. If the blistered stock could be homo- or heterozygous  $bs^{pp}$  (compound with  $bs^s$ ), the crosses might have been (omitting the w marker, segregating as before):

2)  $px\ bs^{pp}/+ \times px\ bs^{pp}/px\ bs^s$  = among px flies in one half all ♀, in the other half many ♀ blist, i.e., about  $\frac{2}{3}$  blist; or

3)  $px\ bs^s/+ \times px\ bs^{pp}/px\ bs^{pp}$  = among px flies half are not blist, of the other half only a part, i.e., about  $+\frac{1}{6}$  blist; or

4)  $px\ bs^s/+ \times px\ bs^{pp}/px\ bs^{pp}$  = among px flies about  $\frac{1}{3}$ – $\frac{1}{2}$  blist.

Table 98 contains the results of twelve such crosses, the first six of which were made with white, the other six with vermillion. The general segregation is the

expected one, i.e.,  $\frac{1}{4}+$ ,  $\frac{1}{4}w(v)$ ,  $\frac{1}{4}px$ ,  $\frac{1}{4}pxw(v)$ . Looking over the  $px$  class, we find (aside from the expected different expressivity of blistering in the two sexes, in the females, on the average, a ratio of less than 2 ♀ blister : 1 not. The individual crosses fall clearly into four groups:

1) No. 8374, with almost all ♀ and  $\frac{1}{2}$  of the  $px$  ♂ blistered, may represent the first type of cross just enumerated as possibilities 1-4.

2) No. 8375, with only a few ♀ and ♂ blistered, may represent the cross type 3.

3) No. 8378, with about  $\frac{1}{3}$  ♀ and few ♂ blistered, may represent type 4.

4) All others, with about  $\frac{2}{3}$  ♀ and many ♂ blistered, may represent type 2.

In the crosses of type 4 also,  $bs^{pp}/bs^{pp} px$  can be produced by crossing over, thus showing the phenotype of  $bs^{pp}$  alone if viable. Actually, 6 ♀ 9 ♂ of this crossover type (c.o.  $px-bs$ ) are found (the reciprocal class being  $px$  and not distinguishable, though they might eventually have been distinguished by low plexation). The phenotype is exactly as described before for  $bs^{pp}$ , namely, a broad web with a blister on top. The percentage of crossing over is rather low. Attention is directed to the strange sex ratios observed in these crosses. They will be discussed below.

In this case the pedigree makes it rather sure that the  $bs^{pp}$  allele had arisen by mutation from  $px bs$  in one individual. In former data we saw this allele occasionally present in the stock. In mass culture it cannot hold its own against  $bs$ . This was shown in the selected stock just described, which contained  $bs^{pp}$  but was, as it seems, not yet completely homozygous since it returned to the condition with little blistering, i.e., without  $bs^{pp}$ , during an interim of  $1\frac{1}{2}$  years when it was not checked while my work was being transferred from Berlin to Berkeley and Dr. C. Stern kindly kept the stocks alive. Later, the same allele  $bs^{pp}$  was once more selected out of the stock by chance. The standard stock had changed to a low blistering. By selection of blistered flies it was returned in a few generations to its old condition, i.e., by selection of modifiers (see above, p. 423). Simultaneously, Mr. M. Kodani made a parallel selection in order to have a good line for salivary work. By chance he hit upon a  $bs^{pp}$  allele and selected a stock with most females and many males blistered, i.e.,  $bs^{pp}$ . There is still another case in which it seems that  $bs^{pp}$  had arisen anew by mutation. In one of the  $px bl$  bottles which had bred true to type for many generations (about 5 years) a breaking up of the type occurred, as described on pages 485 ff., with the production of wild type, low  $px$  without  $bs$ , pointed (the allele  $poi$  and others), and here also  $bs^{pp}$  was found, from which a typical line was isolated which bred true for the described characteristics, and showed also a tendency to an increase of the number of hairs upon the anterior end of the thorax and duplication of the anterior scutellars.

In the presence of the higher allele  $bs^{pp}$ , blistering is caused, so it seems, exclusively by this allele. But in the stock in which this mutant originated we reported an enhancer for blistering in the X chromosome which may still be present in the  $px bl$  line with  $bs^{pp}$ . Crosses with marked X chromosomes made to test this point gave rather strange results, as table 99 shows, for males (females being almost all blistered). In both groups of crosses the crossover conditions and relations of blistering to the X chromosome were tested. The first group (♀ blister 4856 × y cv v f)<sup>a</sup> produced heterozygous females for the X chromosome, the two types of males, and the crossover males. All may recombine with  $px$  and with blistering, as the case may be; the table contains only the males. We notice that blistered males appeared

in almost all classes but in different ratios. In normal males, i.e., without homozygous px chromosome and with the X from the blistered stock, only 1 among 98 was blistered. This is actually a crossover px bs<sup>pp</sup>/bs<sup>pp</sup> ♂ = web blistered. The X chromosome is not involved here. In px males there were 2 among 37, which is a lower percentage than in the stock. In y cv v f males about the same percentage is present (2 in 30), showing, as proved before, that the X chromosome from the blistered stock is not indispensable for blistering. But in the crossover classes the incidence of blistering is much higher than in the noncrossover classes:

Non-c.o. classes px : px blist 63 : 4 = ca. 16 : 1

C.o. classes px : px blist 70 : 13 = 5.4 : 1

There is one crossover class, namely, y cv v, in which px and px blist are about equal (6 : 5). This suggests that though the blistering is predominantly autosomal, something in the X chromosome is also involved and is located somewhere between v and f. This conclusion is brought out also by the group of reciprocal crosses, table 99. Here the ratios are:

Non-c.o. classes px : px blist 52 : 4 = 13 : 1

C.o. classes px : px blist 56 : 12 = 4.7 : 1

y cv v px : px blist 3 : 7

In the same crossover class containing only the right end of the X chromosome, more blistered males appeared than normal ones.<sup>11</sup> The crossover values are hardly informative in view of the large distances involved. They are, with the standard value in parentheses, y-cv = 16.6 (13.7), cv-v = 24.7 (19.3), v-f = 20.5 (23.7).

Thus it appears that the enhancer for blistering was still present in the X chromosome when bs<sup>pp</sup> originated and that it was located at the right end between v and f. But it cannot be as simple as that, as the noncrossover class shows: the intact X chromosome from px<sup>b1</sup> 4856 does not enhance blistering, and neither does the chromosome without the y-containing tip. It seems that it is the absence of the cv-v region of this X chromosome in the presence of a section between v and f which does the enhancing. We remember that in pointed a comparable relation was found, namely, a transposition into the wy region from the left end which could produce by crossing over male lethal classes. In the present case, however, no male lethal class is present and therefore the transposition is ruled out. It may safely be assumed that the sex-linked condition enhancing blistering which had been found in the original stock but could not be localized on account of the paucity of the blistered crossover flies was the same as recorded here, and that one of the reasons for the low incidence of blistering in F<sub>2</sub> was the complicated setup of the enhancer, which the present data show but do not explain.

This sends us back to the crosses reported in table 98, i.e., (px bl bs<sup>pp</sup> stock × white) × px bl ditto and w, and the same with vermilion. In this case, unique sex ratios appeared, never observed in other crosses with the px bl stock, and therefore characteristic for a condition present at the moment that the bs<sup>pp</sup> allele had arisen. In all the crosses in which the paternal X chromosome contained the left end of the original X with the right end replaced (marked by v), the sex ratios are normal, once with a great preponderance of females (see table 100, where the blistered flies have not been separated from px). But in all the crosses in which the right end of

<sup>11</sup> Both y cv v groups added, i.e., 9:12, are statistically significant at and above a 3:1 ratio.

the paternal X chromosome was present with the left replaced by the chromosome marked with w, very abnormal ratios in favor of males are always present, in one case actually 1 ♀ : 4 ♂. In the two cases of the extreme ratios (1 : 3 or 4), all classes,

TABLE 99

Class	(X ple X px bl) <sup>a</sup> No. 7354-66 B	(px bl X X ple) <sup>a</sup> 7353-7447 B	Total	px : px bl
+	97 (+ 1 blist)	122	219	.....
+ px	35	32	67	33.5 : 1
+ px bl	2	..	2	.....
y cv v f	71	78	149	.....
y cv v f px	28	20	48	8 : 1
y cv v f px bl	2	4	6	.....
y	39	22	61	.....
y px	10	6	16	16 : 1
y px bl	1	..	1	.....
cv v f	13	19	32	.....
cv v f px	7	11	18	9 : 1
cv v f px bl	..	2	2	.....
y cv	42	37	79	.....
y cv px	10	8	18	6 : 1
y cv px bl	2	1	3	.....
v f	42	41	83	.....
v f px	15	14	29	14.5 : 1
v f px bl	2	..	2	.....
y cv v	22	27	49	.....
y cv v px	6	3	9	0.82 : 1
y cv v px bl	4	7	11	.....
f	19	36	55	.....
f px	12	7	19	6.3 : 1
f px bl	2	1	3	.....
y f	9	7	16	.....
y f px	2	2	4	.....
cv v	8	2	10	.....
cv v px	1	2	3	3 : 1
cv v px bl	1	..	1	.....
y v	..	1	1	.....
ov f	..	1	1	.....
y cv f	8	5	13	.....
y cv f px	2	3	5	5 : 1
y cv f px bl	..	1	1	.....
v	5	9	14	.....
v px	3	..	3	.....
y v f	..	2	2	.....
y v f px	2	..	2	.....
cv	1	2	3	.....
cv px	1	..	1	.....

+, w, px, and px w, show the low ratio. In the cases of less extreme ratios one or more classes seem to show a 1 : 2 ratio, others being normal, or some show a 1 : 2, others a 1 : 3 or normal ratio without any visible rule except in one case (no. 1), where both classes with w have the low ratio. Undoubtedly, the relation of right half to left half of the X chromosome is responsible and this points again to a trans-

position, which, however, cannot be the left-right transposition found in *poi*, which would produce male lethal classes with the left end without the right end of X. But it is very difficult to visualize how such a transposition could produce female-lethal classes in different combinations with autosomes. There is another possibility, which, however, accounts only for the females of the *px* classes. The salivary glands frequently show a two-band deficiency to the right of *bs* *ba* and sometimes an insertion near the tip of X which might be the same bands. Thus, flies with that insertion might be viable with the homozygous deficiency and not without. Actually, the crossover flies are in favor of this explanation. In the crosses involving an X

TABLE 100  
(Table 98 rearranged)

Group	px		px w(v)		+		w(v)			Sex ratio
	♀	♂	♀	♂	♀	♂	♀	♂		
w group										
1	12	23	8	23	21	21	10	25	2 ♂ + bl web.....	51 : 94 = 1 : 1.8
2	6	34	14	42	12	40	8	41	.....	40 : 157 = 1 : 4
3	13	21	17	18	17	22	15	23	1 ♀ + bl web.....	63 : 84 = 1 : 1.3
4	25	25	15	25	24	30	16	24	.....	80 : 104 = 1 : 1.3
5	13	50	12	32	18	40	9	34	.....	52 : 159 = 1 : 3
6	8	20	12	21	14	27	18	13	.....	52 : 81 = 1 : 1.6
v group										
1	30	18	20	26	22	28	13	26	.....	85 : 98 = 1 : 1.2
2	40	34	52	35	43	37	56	20	.....	191 : 126 = 1.5 : 1
3	28	29	11	22	16	25	27	12	1 ♀ v bl web.....	83 : 88 = 1 : 1.1
4	21	17	15	19	26	17	16	19	1 ♀ 2 ♂ v bl web.....	79 : 74 = 1.1 : 1
5	18	26	22	16	21	24	19	20	3 ♀ 3 ♂ v bl web.....	83 : 86 = 1 : 1
6	20	20	20	23	25	21	20	13	2 ♂ v bl web.....	85 : 79 = 1.1 : 1

chromosome in which the tip marked by *w* has been replaced by crossing over, only not *w* ♀ and ♂ crossovers are found, i.e., flies *px bs<sup>sp</sup> Df/bs<sup>sp</sup>*, X from stock. In the crosses with X chromosomes marked by *v*, i.e., the left end derived from *px bl* present, it is the *v* class which contains the crossovers, i.e., *px bs<sup>sp</sup> Df/bs<sup>sp</sup>*, X left end from stock right end from *v*. But the insertion near *fa* was only found in *poi*, not in *px bl*. It is possible that it was exceptionally present here. But it is of no use to attempt any of these explanations in detail, since further analysis was prevented by the already mentioned circumstances. The data have been discussed in detail in order to show that we must reckon with the presence of many more abnormal conditions of the rearrangement type than can actually be localized.

#### f. THE PHENOTYPES WITHIN THE *px bl* STOCK

The foregoing data have shown the presence of *px* and *bs<sup>sp</sup>* in the *px bl* stock; further, the occasional presence of *bs<sup>sp</sup>*, and also an enhancing condition in the X chromosome which increases blistering as well as plexation. As there certainly is environmental action also, it must be difficult by mere inspection to isolate the genotypes underlying the different degrees of plexation found in the stock. We have described above how some results could be accomplished by crosses with the *Plexates* which,

in some cases, permitted detection of *bs*<sup>rr</sup> in the stock. We report here upon some experiments in which an attempt was made to check upon eventual different alleles of plexus as well as of *bs* and the enhancers. For our test, similar phenotypes from inbred *px bl* were crossed with a standard inbred *px sp* line (a *px sp*). The *px sp* stock was rather homogeneous in phenotype. There was present more or less of a posterior branch of the posterior cross vein (CV), more or less of a vein (EA) in the fifth cell, a small antler attached to the second vein, and a long extra vein in the marginal cell. Only the extremest plus individuals showed a beginning of parallel and periclinal veins. Sometimes the CV appeared to be increased into a little net formation. Seven *F*<sub>1</sub> were studied in detail (2636-42). Two showed a plexus type not distinguishable from that of the a *px sp* stock. In two others approximately half the flies were a *px sp*-like (with respect to plexation). The other half showed a higher grade of plexus with web formation near the cross vein and in II; some individuals had minute blisters (like pearls) above the web in V. One showed in all individuals an intermediate condition between the two types just described. Two more contained half of the low types and half (approximately) of the medium types.

These *F*<sub>1</sub> types were backcrossed again to a *px sp* (2757-69). Five crosses from highest-grade *F*<sub>1</sub> plexus mothers were made, three of which yielded one-half a *px sp* type plexation and one-half the strong maternal type. The latter was present in *px* and a *px sp* flies equally, which indicates that it was not based upon a higher allele of *px* in the *px bl* stock, but upon a modifier in another chromosome or far away toward the left end of the second chromosome. In one more cross the high maternal type was absent, and in another it was very rare. Six backcrosses were made with the ordinary low type of *F*<sub>1</sub>. Four of these yielded only low maternal type, but two gave the same segregation in low and high as the foregoing group (probably owing to transgressing variability in *F*<sub>1</sub>). A few *F*<sub>2</sub> crosses agree in part with these results. In one case the extracted individuals with two *px bl* second chromosomes could not be distinguished from the heterozygotes. One *F*<sub>2</sub> from low *F*<sub>1</sub> reconstituted a low *px bl* type in one-fourth of the *F*<sub>2</sub> flies, namely, generally low plexation but with parallel and periclinal veins. *F*<sub>2</sub> from the high *F*<sub>1</sub>, however, gave one-fourth high plexation, including large webs and some blistered females. In this latter case either a high allele of *px* or a higher allele of *bs* or an enhancer within the second chromosome might have been responsible.

Therefore the same problem was studied by classifying *F*<sub>2</sub> plexus individuals from different crosses with the *px bl* line, the other parent containing neither *px* nor dominant markers nor inversions (which latter can act as intensifiers). We have stated earlier that, as a rule, the *px bl* type is not recovered or only rarely recovered in *F*<sub>2</sub>. In the majority of cases the higher types of plexus formation with parallel and periclinal veins and web are absent in *F*<sub>2</sub>. In order to have a simple classification, we call the *px* type as found in the different standard *px* which we used (description, p. 405) a low plexus; if much plexus formation in the marginal and first cells is combined with a complete periclinal vein but lacks a parallel vein, we speak of medium grade; the addition of more or less of the parallel vein with much web formation is a high grade; and extreme grade, finally, increases the web formation, and has nets at all points of extravenation. There are, of course, all kinds of individual variations, hardly any two individuals being alike. Table 101 gives the results for many *F*<sub>2</sub> in these terms, adding, where noted, the condition of the P indi-

viduals from px bl. (I = low grade; IV = extreme grade.) As there is much transgression, the actual numbers of the classes are not very significant. The tedious classification has been made for over 90  $F_2$  broods by the same recorder (the senior author). In the table certain groups have been lumped together to avoid lengthy details, and only 78 strictly comparable  $F_2$  have been included.

The most conspicuous fact, one which at once becomes evident from the table, is that the enhancer which produces the highest grade of plexus formation in the presence of homozygous px (and the bs allele) is located in the X chromosome. Whereas in all combinations  $F_2$  females and males are more or less alike, with the males, on an average, in lower grades than the females, the  $F_2$  with  $\underline{y}$  grandmothers

TABLE 101

Cross or type of px bl grandparent	not px		px ♀ class				px ♂ class				No. of $F_2$
	♀	♂	I	II	III	IV	I	II	III	IV	
♀ IV blist. Group a	432	312	12	118	2	..	35	54	..	..	8
♀ IV blist. Group b	529	335	..	96	64	..	14	52	17	..	11
♀ IV blist. Group c	821	603	..	142	137	29	16	160	9	2	13
( $\underline{y}$ × px bl ♂ III) <sup>a</sup> ..	614	426	19	148	..	..	..	96	18	7	14
♀ IV.....	169	127	..	26	36	3	21	4	26	..	3
♀ I.....	153	173	59	..	..	..	53	..	..	..	2
♀ III.....	506	451	..	112	55	4	8	122	15	..	7
♀ ♂ IV blist (double reciprocal cross) ..	109	105	..	22	5	..	5	8	1	..	2
♂ IV blist.....	108	103	..	35	..	3	3	46	..	1	2
♂ ? (II, III, IV) a ..	746	535	..	180	61	..	..	145	..	..	10
♂ ? (II, III, IV) b ..	420	323	..	58	83	24	..	56	53	1	6
Total.....	4607	3493	90	937	443	63	155	743	139	11	78

show the males in higher grades than the females. The X chromosome from px bl is therefore needed for the higher grades. If the grandmother was a higher-grade female (grade IV), the  $F_2$  results may be distributed among three groups: a low one (a) with only low grades represented; an intermediate one (b) with the mean between II and III; and a high grade (c) with females of medium and high grades. The double reciprocal crosses which do not produce females with both px bl first chromosomes are of the lower type, as expected. Something in the X chromosome of the px bl, then, enhances the plexus formation.

It would certainly be difficult to disentangle the types completely. But it seems most probable that grades I and II are lacking the enhancer, as the  $\underline{y}$  crosses and the crosses from grade I show. Classes III and IV, then, indicate the presence of the enhancer (probably with differences between homozygous and heterozygous females, which can hardly be distinguished), as the crosses involving grandparental males show, in which no homozygous female may exist (for the X chromosome). But it cannot be excluded that the crosses from type IV grandmothers indicate the presence of the enhancer as well as that of a higher px or bs allele. Much work was done in classifying px grades in crossover classes in crosses with marked chromosomes. The results were so irregular that this type of analysis was discontinued. To mention only one such result: in experiments with X-chromosome markers, both



females and males of class III were found in all crossover classes when only the loci  $y$   $v$   $f$  were involved, though among the males, at least, the majority of marked class III males appeared in classes containing yellow. In crosses involving more markers ( $X^1$ ), males III were found with  $y$   $ec$   $cv$   $v$ ,  $y$   $ec$   $cv$ ,  $y$   $ec$   $cv$   $ct$   $v$   $g$ ,  $y$   $ec$   $v$   $g$   $f$ , and  $y$   $ec$ ; class IV males in the same crosses were found in  $y$   $ec$   $cv$   $f$  and  $y$   $ec$   $cv$   $ct$ .

#### G. OUTCROSSES AND RATIOS OF SEGREGATION

Returning to the problem of lethal classes which were found when  $bs^{pr}$  appeared, we have to study the segregation of the second chromosome marked by  $px$ , after outcrossing  $px$   $bl$  to ordinary stocks. The ratio not  $px$  :  $px$  was calculated for 95 comparable  $F_2$  crosses ( $px$   $bl$   $\times$   $N$ )<sup>2</sup>, separately for females and males. From these ratios

TABLE 102  
F<sub>2</sub> RATIOS NOT  $px$  :  $px$

No. of broods, ♀ and ♂	Ratio														
	-1.2	-1.4	-1.6	-1.8	-2.0	-2.2	-2.4	-2.6	-2.8	-3.0	-3.2	-3.4	-3.6	-3.8	-4.0
♀ .....	1	1	1	3	3	8	7	5	9	5	6	6	6	10	7
♂ .....	..	2	..	1	6	4	8	5	6	3	5	8	10	5	4

No. of broods, ♀ and ♂	Ratio														
	-4.2	-4.4	-4.6	-4.8	-5.0	-5.2	5.9	6.2	6.5	7.7	8	11	14	16	
♀ .....	4	5	5	1	2	3	2	..	1	..	..	..	..	..	
♂ .....	8	2	..	1	2	3	3	2	1	1	2	1	1	1	

the frequency table 102 was derived. Practically all individual broods were good-sized; there were about 15,000 flies altogether. The individual ratios appeared so different that the presence of groups of ratios, 3 : 1 and others, were expected. Therefore the  $\chi^2$  values for all individual broods above 50 individuals were calculated for females and males and the homogeneity test was applied to larger groups. The result was: among the females of 93 broods, 82 fit a 3 : 1 ratio, i.e.,  $P$  for 3 : 1  $> 0.05$ . The remaining 11 broods were tested for 1 : 1, 2 : 1, 4 : 1, 6 : 1, and 10 : 1 ratios. The  $\chi^2$  showed good fit for 1 : 1 in 4 broods, for 2 : 1 in 5 broods, for 4 : 1 in 4 broods, 6 : 1 in 4 broods, and 10 : 1 in one brood. The corresponding data for males were the following. There were 72 broods of sufficient size. Of these, 58 fit a 3 : 1 ratio. Of the 14 remaining broods, 3 fit a 2 : 1 ratio, 5 a 4 : 1 ratio, 10 a 6 : 1 ratio, 11 a 10 : 1 ratio. For the two last-named high-ratio groups the homogeneity test was positive:  $\chi^2 = 4.31$  and 9.75 respectively,  $P > 0.80$  and 0.30. The homogeneity tests for the entire frequency series gave for both sexes (expectation 3 : 1) a significant value  $P < 0.01$ .

Thus we see that the series indicates a mixture of normal and highly divergent ratios, with a considerable amount of transgression. The multimodal aspect of the curve is obviously caused by the presence of different ratios. A 4 : 1 ratio would be expected if a chromosome (the second or another) carried a condition which in the presence of  $px/px$  is homozygous lethal, which would eliminate one-fourth of the  $F_2$   $px$  flies. Further, if a lethal combination were produced by homozygosity of the

px chromosome in conjunction with a recombination of something in the X chromosome, a ratio of 6 : 1 might be produced. If, however, the presence of the px chromosome in heterozygous state leads to lethal recombinations with one or more other chromosomes (say, via hyper- or hypoploidy), the lower ratios of 1, 2, 2.25 : 1

TABLE 103  
SOME INDIVIDUAL RATIOS non px : px

No.	Type of cross	Ratio non px : one px		Number of flies
		♀	♂	
2076	(N × px bl) <sup>2</sup> .....	1.1	1.4	152
2084	(px bl blist × N) <sup>2</sup> .....	1.6	2.0	218
2287	(N × px bl) <sup>2</sup> .....	2.1	1.8	232
2054	(px bl blist × N) <sup>2</sup> .....	2.4	2.5	217
261	(Y × px bl) <sup>2</sup> .....	2.8	3.1	67
792	(px bl × N) × recipr.....	3.1	3.5	259
770	(px bl × N) × recipr.....	3.9	3.6	179
760	(px bl × N) × recipr.....	4.3	4.2	105
300	(Y × px bl) <sup>2</sup> .....	5.1	4.7	106
784	(px bl blist × N) × recipr.....	5.9	7.7	238

may be produced by elimination of not px classes. Finally, if the second chromosome contained a condition lethal when homozygous and not too closely linked with the px locus, all homozygotes for px might be wiped out in a proper F<sub>2</sub> combination, except crossover individuals. Such a situation would account for the very low px ratios above 6 : 1. (A rough calculation assuming a 10 : 1 ratio puts the

TABLE 104  
COMPARISON OF F<sub>2</sub> RATIOS IN FEMALES AND MALES

No.	Type of cross	Ratio non px : one px		Number of flies
		♀	♂	
795	(px bl × N) × recipr.....	2.7	3.7	197
793	(px bl × N) × recipr.....	2.7	5.9	199
291	(Y × px bl) <sup>2</sup> .....	2.8	6.1	104
284	(N × px bl) <sup>2</sup> .....	2.5	16	170
278	(N × px bl) <sup>2</sup> .....	1.8	3.8	209
285	(N × px bl) <sup>2</sup> .....	4.3	2.4	203
2079	(px bl × N) <sup>2</sup> .....	5.2	3.4	209
252	(Y × px bl) <sup>2</sup> .....	3.5	2.2	84
301	(Y × px bl) <sup>2</sup> .....	6.5	2.9	118
493	(N × px bl) × recipr.....	4.0	2.4	215

disturbance at a rather long distance from the px locus). These data, then, encourage a search for a homozygous lethal condition in the second chromosome and eventual translocations between this and other chromosomes.

The detailed data lead one step further still. If male and female ratios are compared in the same brood, we find them frequently alike for high as well as for low ratios. A few selected examples are given in table 103. But still more frequently,

males and females show different or highly divergent ratios. The higher ratio may be on the female or the male side, though more frequently on the male. For a few selected examples see table 104.

This, together with the highest ratios occurring only in males, suggests that the X chromosome also is involved. But it cannot be the case of a simple modifier, as in crosses with attached X females the different types just described are also found. Finally, it is to be noted that out of six cases in which the ratio for females was above 5 : 1, two belonged to a cross with  $\bar{y}$ , i.e., had foreign X chromosomes, three belonged to a combination permitting two foreign X, and only one could not contain females with two foreign X.

An explanation can be found if we remember the two- or three-band deficiency next to *bs ba* and its possible insertion in the X chromosome near *facet*. An  $F_2$  combination of the homozygous deficiency is likely to be combined in the males with half a normal X = lethal and half a duplicated X = viable, which means a ratio for + : px = 6 : 1. In the females the same might be the case, or, if the father had the duplicated X chromosome, all px daughters might be viable. Unfortunately, no known locus seems enclosed in the deficiency near *bs*. The rare cases of extremely high ratios in males (e.g., no. 284 : 65 ♂ + 4 ♂ px = 16 : 1) probably require a different interpretation. It happens that one of the cases with highest ratio (no. 284) was present in an  $F_2$  involving X-chromosome markers, and in this case a deficiency for *echinus* actually was present in one X of the px bl stock (see below). This allows for another lethal class of males except for crossing over. A detailed analysis was not possible, but it suffices to know that the deficiencies and translocations known to occur can account for these ratios in a general way. The important point is the presence of small, genetically demonstrated rearrangements, even though they cannot be located in detail.

#### h. TESTS FOR TRANSLOCATIONS

We have mentioned above certain facts which suggested that small translocations 1→2 and 1→3 as found in pointed, as well as some transposition in the X chromosome, might be present in px bl. Moreover, one small translocation 2→1 was actually found, which eventually accounted for the facts (see p. 345). The standard Patterson translocation test was thus not expected to produce lethal classes except if small duplications were lethal, which is not probable (see above, p. 319). Table 105 contains some of the data of such tests. All crosses were made with a Patterson stock selected for extreme expression of *eyeless*. Actually, in the controls there is very little difference between the ey and not ey classes, the ey class sometimes even being the larger one. But in the px bl II crosses, as before in *poi* crosses, there is such a difference. Looking only at the + and ey class, one might assume that here a part of the ey flies are phenotypically +, as both classes together contain about the same number of flies, e.g., bw and bw ey or e and e ey together. But the extreme difference is found only in the + ey group, not in the others, with the exception of the ♀♀ bw and bw ey in the px bl II crosses, where a huge difference becomes visible. Actually, in a considerable number of individual crosses there were no bw ey females at all; in others, only a single one where ten to fifteen were expected.<sup>12</sup> The obvious explanation is that a third chromosome from px bl II contains a suppressor for ey, as has

<sup>12</sup> Ratios above 8 : 0 and 11 : 1 are significantly different from 1 : 1.

already been discussed for other stocks (see p. 357). The positive tests for this explanation have already been given. The stocks discussed before were all derivatives of px bl. The results are statistically significant. For the Oregon control:

TABLE 105

1-3. CROSS:  $\underline{y}$ , bw, e, ey  $\times$  ( $N \times$  bw, e, ey). 4. CROSS bw, e, ey  $\times$  (px bl II  $\times$  bw e ey)

N	♀							
	Patt	bw e	bw ey	e ey	bw	e	ey	+
1. px bl II.....	105	68	36	82	127	89	69	132
2. px bl 4856.....	52	51	48	47	60	38	36	83
3. Oregon.....	68	73	58	62	87	81	73	89
4. px bl II.....	66	53	58	79	88	83	59	116

N	♂							
	bw e ey	bw e	bw ey	e ey	bw	e	ey	+
1. px bl II.....	67	80	89	85	102	111	80	135
2. px bl 4856.....	58	62	48	43	66	65	44	84
3. Oregon.....	94	123	98	80	85	114	80	123
4. px bl II.....	57	40	43	54	105	58	37	132

$\chi^2 = 12$   $P = 0.10$  for ♀♀ and 29,  $P = < 0.01$  for ♂♂. For px bl II the values are 80.88 for ♀♀ and 52.61 for ♂♂, which are large deviations from normal, far below  $P = 0.01$ . The deviations in the individual classes cannot be said to be confined to the ey group, even though significant differences between the classes are found in the controls

TABLE 106

PERCENTAGE OF DEVIATION FROM NORM = +

	♀						
	Patt	bw e	bw ey	e ey	bw	e	ey
Control.....	24	18	35	30	2	9	18
Expectation.....	20	49	73	38	4	33	48
Difference.....	—4	31	38	8	2	24	30

	♂						
	bw e ey	bw e	bw ey	e ey	bw	e	ey
Control.....	32	..	20	35	31	7	35
Expectation.....	50	41	34	37	25	18	41
Difference.....	18	41	14	2	—6	11	6

also, possibly based upon differential viability in the presence of 0, 1, 2, 3, 4 mutants. In order to compare the px bl II data with the Oregon controls, we might use table 106. The plus type may be taken in both cases as approximating the expected class number. The percentage of deviation from this in the individual classes of the control would measure the loss of viability by accumulation of the recessive markers.

The excess in the experiment after this is deducted would be caused by the respective px bl chromosome present heterozygous in the class, if statistically significant. Aside from the ey situation, this significant difference might show that the different classes have a different viability in different combinations of px bl and foreign

TABLE 107  
Cross: px bl  $\times$  (B, Cy, D, Sc  $\times$  px bl)

B Cy D Sc	B Cy D +	B Cy + Sc	B Cy ++	B + D Sc	B + D +	B ++ Sc	B +++
11	48	4	46	10	37	5	47

+ Cy D Sc	+ Cy D +	+ Cy + Sc	+ Cy ++	++ D Sc	++ D +	+++ Sc	++++
12	41	4	52	11	35	2	69

chromosomes. As we already know that small deficiencies and duplications of different type may be present in such crosses (but without any reciprocal translocations involving lethal deficient or duplicated classes), a result of this type may be expected.

TABLE 108  
Cross (px bl  $\times$  bw, e)<sup>a</sup>

No.	♀						♂					
	+ (6)	e (2)	px (3)	px e (1)	bw (3)	bw e (1)	+ (6)	e (2)	px (3)	px e (1)	bw (3)	bw e (1)
2054	46	15	28	5	16	3	36	15	22	6	20	4
2055	52	7	16	4	25	7	39	5	17	3	15	8
2056	31	13	20	7	10	3	23	7	19	6	15	3
2057	17	3	8	..	7	2	22	2	11	1	3	2
2058	50	13	21	10	19 <sup>a</sup>	4 <sup>a</sup>	32	19	15	2 <sup>a</sup>	11	1
2060	39	13	18	4	24	13	30	11	16	6	22	7
2061	30	12	8	6	15	8	28	10	15	3	12	4
2062	31	8	24	6	19	3	32	14	19	5	8	4
Total	296	84	143	42	135	43	251	83	134	32	106	33

$$\chi^2 \text{ ♀} = 2.82 \text{ } P > 0.70$$

$$\chi^2 \text{ ♂} = 6.69 \text{ } P > 0.20$$

<sup>a</sup> Part ♀, all ♂ Bd.

To gain more definite information many other tests were made. Thus, a cross paralleling the Patterson test was made in which, however, the X chromosomes were exchanged, namely, Patterson test: ♀ both X foreign, ♂ X from px bl, cross bw e ey  $\times$  (px bl  $\times$  bw e ey) = ♀ one X each from both grandparents, ♂ X from foreign stock. The results are found in the fourth column of table 105. They are very similar to the former ones, except that in some respects the result for ♀♀ and ♂♂ are the reciprocal ones of the former case, e.g., first case: difference bw-bw ey, greater in

♀♀; second case: greater in ♂♂, difference + - ey the same; female class bw e ey in first class large, in second small as in ♂♂. This points to a participation of the X chromosome in the deviations.

Many similar tests with dominant and recessive markers have been made, all of them giving comparable deviations from expectation but none of them of a kind to permit clear-cut conclusions. To show only the type of results without going into details, two more examples may suffice. The cross  $px\ bl \times (B, Cy, D; Sc, \times px\ bl)$

TABLE 109  
TEST OF FIT FOR INDIVIDUAL RATIOS FROM TABLE 108

(Ratios and, in parentheses, multiple of standard deviation =  $\frac{Dev}{P.E.}$ . Numbers above 2 = less than 4.6 per cent chance are considered significant. The 1 per cent point is near 2.5. Only tests for more than 50 individuals are included; all others, according to Warwick tables, are insignificant.)

No.	not px : px	not bw : bw	not e : e	+ : e	px : px e	bw : bw e	px : bw	px e : bw e
♀ 2054	2.4 (1.6)	5 (2.9)	3.9 (3.2)	3.1	5.6	5.3	1.8	1.7
♀ 2055	4.6 (2.6)	2.5 (1.3)	5.2 (1.6)	7.4 (3.6)	4.0	3.6	0.6	0.6
♀ 2056	2.1 (2.2)	5.5 (3.0)	2.7 (0.7)	2.4	3.0	3.3	2.0	2.3
♀ 2057	3.6	3.1	6.4	5.7	∞	3.5	1.1	...
♀ 2058	2.8 (0.6)	4.1 (1.9)	3.3 (0.6)	3.8 (1.3)	2.1	4.7	1.1	2.5
♀ 2060	4.0 (1.9)	2.0 (2.9)	2.7 (0.6)	3.0	4.5	1.8	0.8	0.3
♀ 2061	4.6 (2.3)	2.4 (1.2)	2.0 (2.3)	2.5	1.3	1.9	0.5	0.8
♀ 2062	2.0 (2.5)	3.1 (0.4)	4.3 (2.1)	3.9	4.0	6.3	1.3	2.0
Exp.	3 : 1	3 : 1	3 : 1	3 : 1	3 : 1	3 : 1	1 : 1	1 : 1
♂ 2054	2.7 (0.7)	3.3 (0.7)	3.1 (0.3)	2.4 (1.0)	3.7	5.0	1.1	1.5
♂ 2055	3.4 (0.7)	2.8 (0.4)	4.4 (2.2)	7.8	5.7	1.9	1.1	0.4
♂ 2056	1.9 (2.8)	3.1	3.6 (0.8)	3.3	3.2	5.0	1.3	2.0
♂ 2057	2.4	7.2	7.2	11.0	1.0	1.5	3.7	0.5
♂ 2058	3.7 (1.2)	5.7 (3.1)	2.6 (0.8)	1.7 (2.9)	7.5	11.0	1.4	2.0
♂ 2060	3.6 (1.0)	2.5 (1.4)	3.2 (0.3)	3.6	2.7	3.1	0.7	0.9
♂ 2061	3.0	3.5 (0.8)	3.2 (0.4)	2.8	5.0	3.0	1.3	0.8
♂ 2062	2.4 (1.5)	5.8 (3.1)	2.6 (1.2)	2.3	3.8	2.0	2.4	1.2
Σ ♀	3.0 (0.1)	3.2 (1.0)	3.4 (2.1)	3.5 (1.9)	3.4 (1.0)	3.1 (0.3)	1.1 (0.7)	1.0 (0.3)
Σ ♂	2.9 (0.8)	3.6 (2.8)	3.3 (1.6)	3.0 (0.2)	4.2 (2.6)	3.2 (0.6)	1.3 (2.7)	1.0 (0.5)

contains markers for all four chromosomes (Bar, Curly, Dichaete, Scutenicke). The latter has—at least in our stock—a poor penetrance, so that it shows in only about  $\frac{1}{2}$  of the flies. The result of this cross, expecting 16 equal classes, is shown in table 107. The table shows in both sexes alike the classes containing Sc, and the foreign third chromosome (D) to be about  $\frac{1}{2}$  the expected size, i.e., 44 with Sc and 161 without Sc, instead of 102.5 each. But in the presence of the homozygous third chromosomes from  $px\ bl$  the penetrance of Scutenicke is more than twice as much inhibited, parallel to the former case with eyeless, namely, 15 with and 214 without Sc, instead of 114.5 each. Tests for a translocation 4→3, or an attachment of 4 to 3, including the cubitus interruptus test, were negative, but a suppressor action was made probable.

Another example showing the difficulties of simple tests in this case is the following: The crosses involve second chromosomes marked with brown, and third ones with ebony, thus furnishing a simultaneous check upon presence, absence, or heterozygous condition of the second px bl chromosome together with the third in the cross (px bl  $\times$  bw, e)<sup>2</sup>. The normal expectation is 6 + : 2 ebony : 3 plexus : 1 plexus ebony : 3 brown : 1 brown ebony (and eventual crossover flies bw-px). Table 108 shows the results obtained.

In sum total the results look rather normal, thus:

not px : px ♀ 3.01 : 1, ♂ 2.9 : 1

not bw : bw ♀ 3.2 : 1, ♂ 3.6 : 1

non e : e ♀ 3.4 : 1, ♂ 3.3 : 1

(See the  $\chi^2$  values for the entire segregation)

An inspection of the individual broods reveals, however, that this result is not based upon normal sampling, but upon the presence of definite groups of deviation in

TABLE 110  
SEX RATIOS IN DIFFERENT CROSSES WITH px bl

Cross	No. of broods	No. ♀	No. ♂	Average ratio ♀ : 1 ♂
1. px bl $\times$ diff.....	168	12470	7030	1.8 : 1 = ca. 2 : 1
2. px bl pure ♀ ♂ not blist . . . . .	56	4392	4262	1.03 : 1 = 1 : 1
3. px bl pure ♀ ♂ blist.....	22	1943	1789	1.1 : 1 = 1 : 1
4. px bl pure only ♂ blist.....	4	480	495	1 : 1 = 1 : 1
5. px bl pure only ♀ blist.....	46	3942	3656	1.1 : 1 = 1 : 1
6. diff. $\times$ px bl.....	33	1381	1052	1.3 : 1 = ca. 4 : 3
7. $\bar{y} \times$ px bl.....	15	642	491	1.3 : 1 = ca. 4 : 3
Sa.....	344	25250	18775	

both directions, as table 109 shows. In this table the ratios of the individual broods for the different segregating classes are calculated. For each ratio based upon more than 50 individuals the fit has been tested by calculating the multiples of the standard error, i.e., deviation from expectation in terms of the standard error. Values above 2 (4.6 per cent probability of chance) or above 2.5 (less than 1 per cent) may be considered as significantly different from expectation. For groups with less than 50 individuals Warwick's tables were used. As all these latter values were not significant (above 0.005), they have been omitted in table 109. We find significant deviations from expectation, both higher and lower ratios four times for females, once for males in the not px : px segregation; three times for females, twice for males in the not bw : bw segregation; three times for females, once for males in not e : e; once each for females and males in the px : px e groups; and four times the sum total was also significantly different. It is hardly possible to analyze these data in more detail. We discussed before, when analyzing the px segregation, the ratios which might be found and how they could be caused. The same line of argument applies in this case, both for the second and the third chromosomes. As far as they go, the data indicate the presence of such conditions as already analyzed above for the silver alleles and, in addition, special features for the second chromosome as discussed earlier with the segregation of plexus.

## i. THE SEX RATIO IN CROSSES

The results which indicated a transposition within and translocation from the sex chromosome are first checked by a consideration of sex ratios found in crosses of the px bl line with other stocks. These ratios are entirely abnormal. Tables 110 and 111 contain the results for the different  $F_1$  combinations compared with inbreeding the stock. Table 110 contains the summarized results of 344 broods with about

TABLE 111  
NUMBER OF BROODS WITH SEX RATIO n ♀ : 1 ♂

Cross	n														
	0.8	0.9	1	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2	2.1	2.2
1.....	2	5	10	6	11	9	10	11	12	6	7	5	8	3	13
2.....	7	6	16	12	6	5	1	1	2	..	..	..	..	..	..
3.....	1	2	11	3	1	2	1	..	1	..	..	..	..	..	..
4.....	1	1	1	1	..	..	..	..	..	..	..	..	..	..	..
5.....	4	5	17	9	3	5	..	1	..	..	..	..	1	..	..
6.....	2	1	7	5	3	4	3	3	..	1	..	1	..	..	1
7.....	1	..	1	2	2	2	4	1	1	..	1	..	..	..	..

Cross	n														
	2.5	2.6	2.7	2.8	2.9	3	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9
1.....	..	..	1	3	..	9	..	2	3	2	..	3	..	..	1
2.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
4.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
5.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
6.....	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..
7.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

Cross	n														
	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5	5.3	7	7.8	8	8.7	9.7
1.....	..	1	2	..	..	..	1	2	..	2	2	1	1	1	2

44,000 individuals, reciprocal crosses (1, 6, 7), and the possible combinations within the px bl line as controls. In table 111 the range of variation of the sex ratios of individual broods (average size, 130 flies) is tabulated for each group contained in table 110 (1-7), giving the number of broods found, with the respective sex ratios. The first group comprises 168  $F_1$  crosses of px bl ♀ with different wild or mutant stocks, excluding balanced or other complex stocks which might effect the sex ratio by themselves. Table 111 shows that very different sex ratios are present in this group, from normal to complete absence of males ( $\infty$ ). The table further shows that these ratios fall into groups, most of which have an obvious meaning. Very rarely, the males are slightly in excess (ratio below 1) and a normal sex ratio is not very frequent, namely, about 17 per cent of all broods (ratio 0.1-1.1) and part of the 1.2 group. There are two overlapping maxima near 1.3-1.4 and 1.6.



Rough calculation attributes about 17 per cent to each. The next mode is near the ratio 2 : 1, with an estimated 12 per cent of the broods. Another obvious mode lies between 2.2 and 2.3 : 1, containing roughly 14 per cent of the crosses. Another mode lies near 3 : 1, with about 10 per cent of the broods. Whether another small group with about 3.5 : 1 exists is not certain. There are a number of broods with a ratio 4-5 : 1, and finally a group between 7 and 9 : 1. There is obviously a considerable similarity with what we have discussed for pointed, and an even clearer multimodal distribution.

This interpretation derived from inspection of the frequency curve was tested statistically. Again all broods with more than 50 individuals were tested with the  $\chi^2$  test for an expected ratio 1 : 1. All ratios significantly different ( $P < 0.05$  and almost all  $< 0.01$ ) were again tested for expectations 4 : 3, 8 : 5, 2 : 1, 4 : 1, 8 : 1. It is hardly necessary to present all the individual  $\chi^2$  values. We tabulate only the results for  $P = < 0.05$  as limit of significance. There were, in series 1, 147 broods with more than 50 individuals, of which 97 differed significantly from a 1 : 1 ratio; they included all broods beyond a 1.5 : 1 ratio. Of these

5 fit only a 1 : 1 ratio  
 16 fit only a 1 : 1 or 4 : 3 ratio  
 29 fit only a 1 : 1 or 4 : 3 or 8 : 5 ratio  
 5 fit only a 4 : 3 or 8 : 5 ratio  
 31 fit only a 8 : 5 or 2 : 1 ratio  
 6 fit only a 2 : 1 ratio  
 7 fit only a 2 : 1 or 4 : 1 ratio  
 14 fit only a 4 : 1 ratio  
 1 fit only a 8 : 1 ratio  
 1 = 67 : 0 =  $\infty$  fits no other ratio

Arranged according to single ratios :

50 fit a 1 : 1 ratio ranging from 0.8-1.5 : 1  
 81 fit a 4 : 3 ratio ranging from 0.8-2.0 : 1  
 96 fit a 8 : 5 ratio ranging from 1.0-2.8 : 1  
 74 fit a 2 : 1 ratio ranging from 1.4-3.7 : 1  
 22 fit a 4 : 1 ratio ranging from 2.4-4.4 : 1  
 2 fit a 8 : 1 ratio ranging from 5.3-8.7

Thus there can be no doubt that the ratios assumed to occur and visible by inspection do actually occur with an expected amount of overlapping. The simplest case, a mother heterozygous for a sex-linked lethal, i.e., an  $F_1$  ratio 2 : 1, is frequent. Only once the most extreme case was obtained, no  $F_1$  ♂♂, i.e., the mother with sex-linked balanced lethals. The other numbers suggest that different male-lethal combinations are formed by certain autosomal combinations with certain X-chromosome conditions. In other words, the px bl line must be differently homo- or heterozygous for cooperating conditions in sex chromosomes and autosomes, and certain combinations of one or both X chromosomes from px bl mother with normal chromosomes from another stock in the heterozygote must be lethal. A number of the ratios have already been found and analyzed for pointed and are assumed to be based here on the same or similar conditions.

Ratio 1 ♀ : 1 ♂ either normal or the same number of female and male classes lethal.

Ratio 1.33 ♀ : 1 ♂, i.e., about 4 : 3. One of four male classes lethal, i.e., mother heterozygous for a nonreciprocal translocation 1 → autosome, which produces one male class with deficient X out of four.

Ratio 1.6 ♀ : 1 ♂, i.e., about 8 : 5. Three of 8 male classes lethal. This was shown before to be the result of two such translocations, namely, 1→2 and 1→3.

Ratio 3 ♀ : 1 ♂, i.e., 1 female, 3 male classes lethal. The 3 male lethal classes would be the same as before. The female lethal class, one among four, might be caused by duplication 1→2 and 1→3 with double deficiency in 1. If this is correct, the 1.6 : 1 ratio must have a compensation in the females.

Ratio many ♀ : 1 ♂ might be a case of balanced lethals in the mother with considerable crossing over or a comparable situation (see discussion for pointed, pp. 311 ff.).

Thus we expect that the px bl stock already contains all the small translocations found in pointed or others of the same type, in addition to other abnormal conditions, and that different individuals of the stock may have one or the other or all of these rearrangements.

TABLE 112  
FERTILITY TEST

Cross	Fertile among 200	Per cent fertile
♀ px bl A × ♂ Oregon.....	148	74
♀ Oregon × ♂ px bl A.....	141	70.5
♀ px bl B × ♂ Oregon.....	149	74.5
♀ Oregon × ♂ px bl B. ....	134	67
Control Oregon pure.....	171	85.5

The other control crosses seem to have a simple 1 : 1 ratio with unimodal distribution. The groups numbers 5, 6, 7 were tested statistically by calculating  $\chi^2$  for each brood of more than 50 individuals. The results were:

Series 5: 44 broods, 42 fit a 1 : 1 ratio, 2 differ

Series 6: 26 broods, 22 fit a 1 : 1 ratio, 4 differ

Series 7: 14 broods, 11 fit a 1 : 1 ratio, 3 differ

Of the two significantly differing broods of series 5, one had an exact 2 : 1, one a 4 : 3 ratio. The four of series 6 all had good 2 : 1 ratios, and of the three of series 4, two looked like 4 : 3 and one like 2 : 1. The 2 : 1 ratios indicate occasional sex-linked lethals with unimodal distribution. Rare 1 : 3 ratios in the pure-stock crosses would indicate an occasional unbalance within the stock which otherwise must be completely or almost completely balanced, which means that whatever lethal classes may be formed die in equal numbers in both sexes (though different classes may be lethal in the two sexes). If this is true, matings within this line ought to produce fewer offspring than do controls. We first tested the fertility both of males and of females of two different px bl stocks in reciprocal crosses of 200 pairs each under identical conditions. Table 112 contains the results. This shows a small increase of sterility in the px bl crosses.

In order to gain an idea of the amount of lethal classes in this balanced line the following experiment was carried out. From one of the px bl inbred stocks, 20 ♀♀ with blisters and 20 ♀♀ without blisters were crossed to Oregon ♂ (columns 1, 2 of table 113). Further, 20 females from the same bottles were mated to their brothers, and 20 Oregon pairs served as controls. Females of about the same size, all from fresh bottles, were used, all conditions were kept as equal as possible, and the counts were made on the seventeenth day of breeding at  $25^\circ \pm 1^\circ$ . Table 113 shows the

result, which is perfectly consistent throughout. It shows, aside from the sex ratios already discussed, that px bl females crossed to wild-type males produce even more offspring than the control (heterosis). The pure px bl produce (only the females are strictly comparable) less than half of the daughters found in the offspring of their cross-bred sisters and about  $\frac{2}{3}$  of the number in the controls. This is expected when lethal classes are produced in the balanced stock; they might amount to about half of the individuals.

Returning again to table 110, we find as no. 6 the reciprocal cross from no. 1, the difference being that the same autosomal conditions are combined with an always normal X in the male. We expect, therefore, lethal classes only with autosomal combinations not balanced to the foreign X (if any) and, therefore, normal sex ratios, except a few cases of a 3 : 1 ratio, as explained above. Finally, a smaller number of broods is tabulated as no. 7, the crosses of females with attached X chromosomes to px bl males. The males always have only one type of X from the px bl father, the females no such X. A mode near 1.3 was found, which may or may not be significant. Sex ratios are sometimes irregular in attached X crosses which involve *per se* lethal classes (XXX and YY).

#### j. TESTS FOR TRANSLOCATIONS AND TRANSPOSITIONS

As the greatest complications were expected in the first chromosome, judging from the variation in the  $F_1$  sex ratios reported above, numerous crossover tests were made and repeated at different times. The first group to be reported tests px bl in different combinations with y v f. Instead of using the simple backcross ( $\text{px bl} \times \text{y v f}$ )  $\times$  y v f, different types of  $F_2$  were used which behave like backcrosses as far as the X chromosome is involved but furnish in addition all possible recombinations of the autosomes, including the homozygous condition for the different px bl chromosomes (only the second being marked by px). Thus, many more possibilities of discerning lethal classes are given. The cross ( $\text{y v f} \times \text{px bl}$ )<sup>2</sup> thus tests the male X chromosome and furnishes crossover classes in both sexes. The reciprocal cross ( $\text{px bl} \times \text{y v f}$ )<sup>2</sup> tests the female X chromosome with only male crossover classes. The double reciprocal cross ( $\text{px bl} \times \text{y v f}$ )  $\times$  ( $\text{y v f} \times \text{px bl}$ ) again tests the female X chromosomes both with female and with male crossover classes. Tables 114–116 contain the results of this first set of crosses. Table 114 shows at once that special and not simple features are present.

One feature of table 114 is that among the males the forked class is absent five times (against 47 in the reciprocal classes); four times it is very small, with 10 flies as against 50 in the reciprocal classes; seven times it is about one-half the size of the corresponding class (27 to 59); and only three times is it about equal (all values are significant). In two of the male groups without f flies, the v f group is also missing; once, only 1 individual (against 9 y) are present; and twice, one-third to one-half of the reciprocal class are present.

Our former analysis of pointed indicates in which direction an explanation may be found. If a nonreciprocal translocation from the first to the second and third chromosomes of the type found in pointed were present, deficient and lethal male classes could be produced in  $F_2$  with a deficient X recombined with nonduplicated autosomes. The presence of the autosomal duplication depends upon the constitution of  $F_1$ , and this again upon absence, heterozygosity, or homozygosity in the px bl

parent. Thus  $F_2$  may be  $Dp2/+ \times Dp2/+$ ,  $Dp2/+ \times +/+$ ,  $+/+ \times +/+$ , and eventually, the same combinations simultaneously with  $Dp3$  ( $Dp$  from  $T\ 1 \rightarrow 3$ ). With the proper deficient crossover class in the first chromosome, therefore, different ratios of lethal classes are possible, namely, for each of the deficiencies  $1/4$ ,  $1/2$ , or all lethal, i.e., ratios of the reciprocal classes of 4 : 3, 1 : 1, or  $n : 0$ . In the presence of both deficiencies the ratios are 4.4 : 1, 3.3 : 1, 3 : 1, and enhanced chances for  $n : 0$ . Table 114

TABLE 113  
FERTILITY TEST

Mating	px bl, blist $\varphi \times + \sigma$		px bl, $+ \varphi \times + \sigma$		px bl pure $\varphi$ and $\sigma$		+ control	
	$\varphi$	$\sigma$	$\varphi$	$\sigma$	$\varphi$	$\sigma$	$\varphi$	$\sigma$
1.....	115	53	152	80	51	22	16	17
2.....	58	17	104	36	92	60	70	84
3.....	63	28	117	74	36	40	92	112
4.....	112	52	149	53	27	21	9	26
5.....	94	71	127	85	36	38	79	78
6.....	40	39	135	80	21	19	60	88
7.....	115	56	121	49	25	18	97	93
8.....	109	25	105	57	17	13	72	61
9.....	116	72	118	58	36	16	98	83
10.....	139	71	163	37	67	52	88	87
11.....	77	44	81	51	84	58	76	46
12.....	35	12	84	61	13	8	121	98
13.....	94	32	122	75	12	10	72	70
14.....	109	70	123	78	88	70	62	55
15.....	83	35	47	52	84	59	87	99
16.....	99	64	78	64	57	38	88	83
17.....	123	74	91	45	94	71	53	56
18.....	100	50	118	62	24	26	86	70
19.....	132	90	97	65	69	73	28	26
20.....	90	88	108	50	46	41	70	63
Sa.....	2003	1043	2240	1212	979	753	1424	1395
M.....	100	52	112	61	49	36	71	70

shows that the ratios of  $\sigma$   $yv-f$  are rarely more or less normal and might fall into the ratios just mentioned. This would mean that the two deficiencies are located left of  $f$  and that different recombinations with the autosomal duplications are found. Statistical tests for the reciprocal male classes  $yv-f$  were made for the individual crosses (all fewer than 50 individuals), using Warwick's tables; for the sum totals, by calculating multiples of deviation in terms of standard error, values above 2 (or 2.5) being considered significant. Out of 17 individual cases 8 were significantly different from a 1 : 1 expectation. All but one of these (the 13 : 0 ratio) fitted as well a 3 : 1 as a 4 : 1 ratio, though the 3 : 1 ratio gave consistently lower deviations from expectation. The sum total gave extreme discrepancy from 1 : 1 expectation ( $d/PE=12.02$ ) and good fit for 3 : 1 or 4 : 1 ( $d/PE=1.39$  and 1.25 respectively). This shows the adequacy of the interpretation. The two reciprocal classes  $y-vf$  also show discrepancies (see the sum total, table 114, and details,



TABLE 115  
CROSSOVER VALUES TOGETHER WITH THE RATIOS OF THE TWO RECIPROCAL CLASSES (EXPECTATION 1:1) IN THE CROSSES OF TABLE 114

No.	Crossover				Ratios of reciprocal classes							
	♀		♂		+ : y v f		y : v f		y v : f		v : y f	
	y-v	v-f	y-v	v-f	♀	♂	♀	♂	♀	♂	♀	♂
390	40	21.2	27.8	26.2	1:1	1:2	0:28	1:1	11:1	4.3:1	1:2	1:5
391	28.7	12.1	20.6	28.2	1.7:1	1:1.4	1.6:1	1.4:1	6:1	7:1	1:0	1:1
494	28.6	28.6	28.4	22.8	1.3:1	1:1	1:1	1:1	1:1	1:1	1.5:1	1.3:1
396	....	....	23.7	23.7	....	1:1.3	....	4.5:1	....	4.5:1	....	0:3
406	....	....	22.5	19.7	....	1:1.3	....	11:0	....	9:0	....	0:5
407	....	....	35.6	20.8	....	1:1	....	2.7:1	....	11:0	....	1:4
461	....	....	30.9	20.6	....	1.4:1	....	1:1	....	1.5:1	....	1:1
414	32.8	26.2	31.6	23.7	1:1.4	1:1	1:1.7	9:1	1:1.4	7:0	3:1	1:1
433	22.1	31.2	40.3	29.9	1:1.6	1:1.6	0:6	1.5:1	2.2:1	1.6:1	1:4.5	1:5
444	27	18.3	42.2	15.6	1:1	1:1	2.5:1	2:1	1:1.3	7:0	0:1	1.3:1
445	26.7	28	20.8	28.6	1:1	1.2:1	1:1	7:0	1:1	13:0	1:5	1:1
448	35.2	28.6	33.3	25.3	1.3:1	1:1.2	1:2	1:1	4.3:1	4.3:1	2:1	1:1.5
492	33.3	16.7	33.3	18.9	1:1	1:1.7	1:1.2	2.6:1	1:1	2:1	2.5:1	1:4
493	....	....	39.4	20.2	....	1:1.2	....	1.3:1	....	3:1	....	1:3
416	....	....	22.9	18.8	....	1:1	....	2.3:1	....	3:1	....	1:0
417	....	....	31.9	20.9	....	1:1.4	....	4.6:1	....	2:1	....	1:0
418	....	....	27.5	21.7	....	1:2	....	1.3:1	....	2.3:1	....	1:1.5
452	....	....	25	15	....	1:1.5	....	4:1	....	3.6:1	....	....
453	....	....	30.1	19.2	....	1:1.7	....	1:1	....	2.7:1	....	2:1
Σ	30.7	23.6	31.1	22.7	1:1	1:1.2	1:1.5	2:1	1.7:1	3.6:1	2:1	1:1.8

Standard: y-v = 33; v-f = 23.7.

TABLE 116  
SEGREGATION OF PX IN THE CROSSES OF TABLE 113

No.	Ratio not px : px ♀ in classes							Ratio not px : px ♂ in classes						
	+	yvf	y	yf	v	vf	v	+	yvf	y	yf	vv	f	v
390.....	17:2	13:7	.....	18:10 <sup>a</sup>	8:3	0:1	2:0	9:6	18:12	6:3	7:1	7:6	2:1	1:0
391.....	17:8	12:3	9:4	3:4	4:2	1:0	1:0	9:5	12:8	8:2	7:0	11:3	2:0	1:1
394.....	13:7	13:2	5:3	6:1	5:3	3:4	3:0	18:7	19:6	8:1	6:3	6:1	5:1	3:1
396.....	57:23 <sup>b</sup>	.....	.....	.....	.....	.....	.....	16:4	15:4	5:4	0:2	7:2	2:0	.....
406.....	49:23 <sup>c</sup>	.....	.....	.....	.....	.....	.....	14:13	18:9	15:4	5:2	9:2	.....	.....
407.....	69:32	.....	.....	.....	.....	.....	.....	13:9	11:4	6:2	6:3	6:0	2:2	1:1
461.....	73:19	.....	.....	.....	.....	.....	.....	?	8:1	8:1	?	5:2	.....	1:0
414.....	10:2	10:4	4:2	0:1	3:1	5:2	2:1	?	16:3	14:2	9:2	11:0	6:1	1:0
433.....	17:1	21:8	.....	4:2	6:1	4:0	2:0	?	17:3	17:3	9:2	6:1	.....	3:1
444.....	17:7	16:6	12:3	6:0	5:1	6:2	.....	14:8	21:2	5:2	.....	9:4	.....	3:0
445.....	17:7	17:4	9:0	6:2	7:2	6:3	1:0	20:7	18:3	9:3	8:3	10:4	3:0	1:1
448.....	15:9 <sup>d</sup>	12:6	6:2	10:5	12:2	2:1	4:2	9:5	16:5	9:3	8:3	6:2	4:0	1:0
492.....	29:7	26:5	11:4	12:6	5:2	6:0	5:0	13:5	22:8	18:0	12:3	9:3	1:3	2:1
493.....	.....	.....	.....	.....	.....	.....	.....	15:5	20:5	16:3	12:3	5:1	1:1	.....
416.....	62:20 <sup>e</sup>	.....	.....	.....	.....	.....	.....	11:4 <sup>f</sup>	14:0	6:1	3:0	5:1	3:3	0:1
417.....	77:25 <sup>g</sup>	.....	.....	.....	.....	.....	.....	13:5	19:7	17:6	5:0	8:4	3:0	1:1
418.....	74:23	.....	.....	.....	.....	.....	.....	10:3	21:6	4:4	4:2	5:2	3:0	1:1
452.....	57:22 <sup>h</sup>	.....	.....	.....	.....	.....	.....	11:3	20:2	11:1	0:3	6:1	2:0	.....
453.....	59:28 <sup>i</sup>	.....	.....	.....	.....	.....	.....	10:5	20:5	6:3	7:3	6:2	3:0	0:1
Σ.....	729:271	140:45	56:16	74:31	55:17	33:11	20:3	214:90	329:95	185:50	92:32	139:43	39:12	23:7
	2.7	3.1	3.5	2.4	3.2	3.0	6.7	2.4	3.5	3.7	2.9	3.3	3.3	3.3
d/PE for Σ.....	2.3	0.25	0.81	1.69	0.4	0	2.1	2.76	1.8	2.01	0.3	0.76	0.48	0.63

<sup>a</sup> All extreme  
<sup>b</sup> 2 blist.  
<sup>c</sup> 2 blist.  
<sup>d</sup> 2 blist.  
<sup>e</sup> 4 blist.  
<sup>f</sup> 8 blist.  
<sup>g</sup> 4 blist.  
<sup>h</sup> 4 blist.  
<sup>i</sup> 4 blist.

table 115) in favor of the *y* class. The ratio 217 : 110 is significantly different from 1 : 1 ( $d/PE = 8.9$ ). This indicates the location of one of the deficiencies between *y* and *v* and probably nearer to yellow. Correspondingly, we find here many more low ratios, but also the high ratios up to  $n : 0$  for *y* : *vf* ♂. Altogether, the facts for males agree with the expectation and the presence of the same or similar translocations 1→2 and 1→3 as found in *poi*.

In the females we may expect normal ratios because one of the *X* always contains the normal loci. The numbers are smaller; they are, leaving aside the first cross no. 39, for *yv* : *f* = 64 : 45 = 1.4 : 1; ( $d/PE = 2.86$ ) for *y* : *vf* = 72 : 77 = 1 : 1.

But this cannot be the entire solution. *F*<sub>1</sub> males clearly cannot survive with a deficient *X* without autosomal duplication (cross *px bl* × *N*). Therefore either all *F*<sub>1</sub> males must have been *Dp*/(*Dp* 2 or 3 or both), or in the *X* chromosome the formerly studied transpositions of the same loci must have been present to compensate for the deficiencies. The normal *F*<sub>2</sub> male class ought to answer this question. If it contained the deficiency (or deficiencies) its ratios ought to be the same as in the *f* class, which is not the case. The ratios + : *yvf* ♂♂ are in most cases normal, but sometimes are as 1 : 2. But this 1 : 2 ratio or the normal ratio does not coincide with the same ratio for *yv* : *f* ♂. Therefore another complication must be present, which might well be the transposition. In view of the long distances involved and the few markers, a further inquiry will be useless for this peculiar cross. The crossover values (see table 115) do not contain any pertinent information, but the summarized ratios for + : *yvf*, in which a lower viability of the multiple recessive chromosome ought to work in favor of the + groups is 317 : 409 with a highly significant  $d/PE = 5.08$ .

We turn now to the abnormal brood no. 390 (first in tables 114–116), which contains no *y* class among the females, whereas the reciprocal class *v f* contains 28 females. The high number of this class—compared with other broods of about the same size,—together with the high crossover value 40 (standard 33), demonstrates that this is not a normal feature. As table 116 shows, all the 10 *px* females among 28 females exhibit an extreme plexus formation, which otherwise occurs only in 3 more individuals among the hundreds of plexus flies. We shall see later that such an extreme *px* may characterize an *ec* deficiency. The whole situation points out that these excess *v f* females are actually the missing *f* ♀ with a vermilion deficiency. They turned out to be sterile (as are the *ec* deficiencies). This prevented further analysis. This is to be regretted, as other features were present which require a very complicated situation, e.g., all 10 extreme *px* females must have been the deficiencies. But where are the other *px* segregants? The *y* class is here completely missing, but the other classes containing *y* are normal. No explanation covering all these facts can be offered, and a similar result did not occur again. It is possible that the missing yellow females are contained in the relatively large *yv* class, i.e., *y* and *v*-*Df*. But here no extreme *px* was found. Unfortunately, the *yv* class had not been tested further. We shall see below that an *ec* deficiency has been found in the stock. Thus all we can say is that additional abnormalities may be present in *px bl* involving loci of the *X* chromosome and also *px*.

In these crosses the second chromosome of *px bl* was marked with *px bs*, which might furnish information concerning the supposed 1→2 translocation. Table 116 contains the data for the *px* segregation in the different crosses and classes found



in table 114. Though some regularities are visible, e.g., in both sexes lower ratios for + and v f than for the reciprocal y v f and y, no clear conclusion can be drawn. The statistical test (see lower end of table) shows only few ratios which might be significantly different from a 3 : 1 ratio. They are the + and v classes in females and the + y classes in males. This does not allow of definite conclusions. We notice again, as before, that blistering occurs only in normal females with two X from px bl.

TABLE 117  
(px bl × X<sup>r</sup>) × X<sup>r</sup>

Class	♀	♂	d/FE
+	42	24	3.29
y w ec cv ct v f.	25	2	16.8
y	..	..	....
w ec cv ct v f.	..	..	....
y w	5	1	All left of ▼ 21 : 14 = 2.0 All right of ▼ 38 : 15 = 7.72
ec cv ct v f.	..	..	
y w ec	2	4	
cv ct v f.	7	..	
y w ec cv	6	5	
ct v f.	5	..	
y w ec cv ct	8	4	
v f.	10	1	
y w ec cv ct v	13	6	
f.	13	4	
y w	2	..	All left of ▼ 21 : 14 = 2.0 All right of ▼ 38 : 15 = 7.72
y w ec cv ct f.	1	..	
v	3	1	
ec cv	1	..	
cv ct	1	2	
cv ct v	4	7	
ec cv ct	..	1	
ct v	3	..	
y w ec	1	..	
v	1	..	
	152	56	9.8

Out of many crosses made with more first chromosome markers, only one gave a result comparable to those contained in table 114, a cross with seven recessive markers (X<sup>r</sup>). As table 117 shows, almost  $\frac{3}{4}$  of the males were lethal. In the + class about half of the males are missing, in the X ple class most of them. Further, all males are missing when the right half of the X chromosome, including cv, is replaced, and most of them if v f or f is present. Clearly, a region of the X chromosome near vermilion is needed for male survival in the presence of the left half of the original X. The last column of the table contains the statistical tests for an expected 1 : 1 ratio with significant values except for the crossover classes left of v. A transposition alone does not explain all data, which suggest, as before a combination of a transposition with translocations 1→autosomes.

When we tried to analyze this condition further, it turned out that the long-inbred stocks no longer contained the same abnormalities, which it seems had been inadvertently selected out while other work was at the fore. A series of 44 F<sub>2</sub> be-

tween px bl and N (an X ple stock with 9 markers) gave more normal than abnormal sex ratios, but never gave the same features as reported above. Table 118 summarizes only the grand total since the individual broods do not furnish any decisive information. The absence or small number of multiple recessive classes is obviously based on viability. Taking this into account, reciprocal classes are about equal.

TABLE 118  
FORTY-FOUR CROSSES (px bl  $\times$  X<sup>9</sup>)  $\times$  (X<sup>9</sup>  $\times$  px bl)

Class	Sum total		d/PE
	♀	♂	
+	1703	1360	9.2
X <sup>9</sup>	229	217	0.8
y	48	65	2.2
w ec cv ct v m g f	.....	.....	.....
y w	118	117	0.2
ec cv ct v m g f	2	1	.....
y w ec	221	161	4.5
cv ct v m g f	136	66	7.3
y w ec cv	173	194	1.6
ct v m g f	153	134	1.8
y w ec cv ct	253	240	0.8
v m g f	280	224	3.7
y w ec cv ct v	73	48	3.2
m g f	62	65	0.3
y w ec cv ct v m	60	30	4.7
g f	114	113	0.2
y w ec cv ct v m g	62	54	1.1
f	260	182	5.5
All multiple c.o. y not w	11	25	P = 0.029
All multiple c.o. w not y	3	3	P = 1.0
multiple c.o. w-ec	110	53	6.7
multiple c.o. ec-cv	121	120	0
multiple c.o. cv-ct	109	110	.....
multiple c.o. ct-v	142	115	2.4
multiple c.o. v-m	101	59	4.9
multiple c.o. m-g	94	85	1.1
multiple c.o. g-f	197	180	1.2
Total no. of individuals	4835	4021	12.8

The statistical tests show significant aberrations from a 1:1 sex ratio in favor of females (last column, table 118) in the + class; further, among single crossover classes in y w ec, ec ct v m g f, v m g f, y to v, y to m, f; among multiple crossover classes, in those with a break between w-ec, ct-v, v-m. The exact ratios are not relevant, as they are based upon many broods which probably were not alike genetically since the px bl parent might have introduced any of the aforementioned abnormalities or none. The regions involved in the aberrant ratios are those mentioned before. Here, likewise, a simple explanation is out of the question, and both transpositions within the first chromosome and translocations into an autosome are required to account for the facts. A check for the second chromosome marked by

px was made, but without clear results. Crossing over is rather normal except for a rather high value in the ec-ct region.

This experiment furnishes some additional data for problems already discussed, namely, the segregation of blistering and plexation. The results are in conformity with those presented above and are as follows. Out of 44 broods only 7 contained altogether 28 ♀ 1 ♂ blistered. These were found exclusively in the + class, though about  $\frac{1}{3}$  of the females and  $\frac{3}{4}$  of the px males belonged to the not + classes. The

TABLE 119  
SEGREGATION OF px IN TABLE 118

	Ratio not px : px															
	below 1:1	1-2:1	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	14	17	27
Cases ♀ .....	1	5	9	16	5	3	2	..	..	..	..	..	..	..	..	..
Cases ♂ .....	..	2	7	9	3	3	9	2	2	..	..	1	1	1	1	1

presence of the entire px bl X chromosomes and therefore probably of more than one locus is needed for the blistering effect. The range of variation of not px : px ratios in 42  $F_2$  is shown in table 119. The ratios closely resemble those which we tabulated before (see table, p. 456) for other  $F_2$  crosses as a whole and therefore will not be analyzed further.

A further attempt was made to localize the small translocations expected to be present in px bl on the basis of the foregoing data by crossing px bl on a rather large scale with a number of available stocks marked with many recessives in two

TABLE 120  
(px bl  $\times$  a sp)  $\times$  a sp

	♀	♂
+	465	470
a.sp.	449	246
a.	30	21
sp.	31	23

Crossover, 6.2 ♀ 5.8 ♂; stand., 7.8.

or three chromosomes. The tedious counts gave practically no results because lethality or unviability of classes with many homozygous markers in more than one chromosome obscured any possible clear information; and further, the numerous classes—in one experiment with 3 marked chromosomes, 84 discernible classes—could not contain sufficiently large numbers to give significant information. Thus, these data tend to show only the difficulty of genetically testing small transpositions and translocations not involving known loci and probably not lethal in duplicated or deficient condition except for deficiencies in the male first chromosome.

More successful were tests for marked regions in one chromosome alone. One test which was carried out in a series of simultaneous crosses for the right end of the second chromosome marked with a sp gave very similar results to those previously

described for pointed, though pointed is not present, as is shown clearly by the lack of sp suppression. The ratios are found in table 120. The a sp sex ratio of 2 : 1 suggests again the presence of a small translocation 1→2 into the a-sp region. Years later, many more such tests were made with the same or different markers. Nothing

TABLE 121  
(px bl × h cu) × h cu

	♀	♂	Sex ratio
+. . . . .	296	269	1.1 : 1
h cu. . . . .	180	80	2.3 : 1
h. . . . .	69	56	1.2 : 1
cu. . . . .	57	55	1 : 1
	602	460	1.3 : 1

comparable was found, showing, as before, that within the stock the presence of the small rearrangements was in constant flux. We shall return to the second chromosome later.

Very similar were the results for the third chromosome. In a considerable number of crosses no lethal action for males was found which would point to a nonreciprocal translocation 1→3. But sometimes a positive result appeared. Thus in a set of

TABLE 122  
(px bl × e tx) × e tx

	♀	♂	Sex ratio
8 normal broods:			
+. . . . .	376	336	1.1
e tx. . . . .	204	182	1.1
e. . . . .	96	84	1.1
tx. . . . .	76	69	1.1
6 broods with obviously the translocation 1→3:			
+. . . . .	335	257	1.3
e tx. . . . .	153	69	2.2
e. . . . .	64	32	2.0
tx. . . . .	62	36	1.7

crosses (px bl × ru ve h th) × ru ve h th, perfectly normal results appeared among 470 ♀ 542 ♂, but for unusually high crossover values (e.g., ru-ve standard 0.2, obtained 2.4). But in another series exploring the region near h the result was different, as table 121 shows. The table shows one-half of the males with the foreign third chromosome lethal but the h cu region not involved. In pointed we had found a region beyond the ebony region, far to the right in the other arm, responsible. Actually, the same region turned out to be decisive here, too. The following results obtained from a series of crosses with marked third chromosomes, including ebony and made in different years, are given in table 122.

The latter group in table 122 indicates the location of the insertion to the right of ebony. Another series testing the region to the left of ebony is in agreement,

namely, (px bl × ru h cu) × ru h cu, curled (cu), being 20 units left of e. The cu-containing class may therefore be without ebony and the region right of e by double crossover (table 123). Altogether, we have reason to assume that the same or similar translocations and transpositions as were found in poi are distributed also within the px bl stock.

TABLE 123  
(px bl × ru h cu) × ru h cu

	♀	♂	Sex ratio
5 normal broods:			
+.....	121	151	0.8
ru h cu.....	56	52	1.1
All c.o. no cu.....	80	85	0.9
All c.o. with cu.....	53	47	1.1
6 broods with translocation:			
+.....	244	231	1.1
ru h cu.....	139	73	1.9
All c.o. no cu.....	183	171	1.1
All c.o. with cu.....	100	74	1.4

TABLE 124  
px bl Crosses Showing AN ec-Df

No.	Cross	♀+	♂	♀ ec	Remarks
1708	px bl blist × X <sup>a</sup> .....	79	51	1	ec with short bristles, sterile
1711	px bl blist × X <sup>a</sup> .....	17	9	2	All ec short bristles, sterile
1715	px bl blist × X <sup>a</sup> .....	91	65	6	All ec short bristles, sterile
585	px bl × X <sup>a</sup> .....	20	15	1	All ec short bristles, sterile
....	22 more similar crosses.	Normal		..	
733	(px bl × X <sup>a</sup> ) <sup>2</sup> F <sub>1</sub> no ec....	65	52	1	♀ ec extreme px and bb
	Many the same.....	Normal		..	
748	585 <sup>2</sup> .....	130	30	2	2 ♀ ec, 1 extreme px and bb and blist, 1 not px
749	585 <sup>2</sup> .....	82	43	1	♀ ec extreme px and bb
750	585 <sup>2</sup> .....	56 (8)*	23	22	4 ♀ ec extr. px bb, 18 not px
751	585 <sup>2</sup> .....	52 (5)	30	6	5 ♀ ec extr. px bb, 1 not px
752	585 <sup>2</sup> .....	Normal		..	
753	585 <sup>2</sup> .....	53 (6)	37	20	2 ♀ ec extr. px bb, 18 not px
754	585 <sup>2</sup> .....	131 (25)	53	1	1 ec bb but not px
756	585 <sup>2</sup> .....	58	2	2	Very few males. 1 ♀ ec extr. px bb, 1 not px
490	(px bl × X <sup>a</sup> ) <sup>2</sup> F <sub>1</sub> no ec....	96 (19)	68	11	All ♀ ec extr., px bb
491	(px bl × X <sup>a</sup> ) <sup>2</sup> F <sub>1</sub> no ec....	101 (21)	50	4	All ♀ ec extr., px bb

\* No. in parentheses px.

#### k. DEFICIENCIES IN px bl

As the salivaries showed that quite a number of small deficiencies were present in the px bl stock, the regions in question, as well as many others (about 200 loci), were tested for the presence of deficiencies at known loci, and the tests were also carried on another generation to test deficiencies by translocation. The results were always negative, with one exception, the echinus locus (1, 5.5). Deficiencies for this locus

were always found when a number of crosses involving the marker *ec* were made. Table 124 contains all the cases found in  $F_1$  and  $F_2$ .

In about 15 per cent of the crosses in which a check for an echinus deficiency in  $F_1$  was made, a few *ec* females appeared, altogether 10 among 207 normal sisters. All of them had short bristles like hobbled or Minute (no *bb* was present in the test stocks) and were sterile, though they lived up to two weeks in the mating bottles.  $F_2$  from normal broods gave, in a considerable number of cases, only normal offspring, but once (no. 733) an *ec* female appeared, hobbled, sterile, and extreme plexus. From one of the  $F_1$  which had contained one echinus female (no. 585), 8  $F_2$

TABLE 125  
(*px bl*  $\times$  *sc ec ct*)  $\times$  (*sc ec ct*  $\times$  *px bl*)  
(♀ only)

Class	No. 760	No. 761	No. 763	No. 764	$\Sigma$
+	42	37	40	38	151
px	10	8	8	8	34
<i>sc ec ct</i>	19	6	8	7	40
<i>sc ec ct px</i>	3	1	..	1	5
<i>sc</i>	1	..	..	1	2
<i>sc px</i>	..	..	1	..	1
<i>ec ct</i>	1	..	3	..	4
<i>ec ct px</i>	1	1	1	1	4
<i>sc ec</i>	11	13	1	6	31
<i>sc ec px</i>	2	..	2	5	9
ct	6	4	1	3	14
ct <i>px</i>	1	1	1	..	3
ec	5	1	5	5	16
ec <i>px</i>	3	2	6	4	15
<i>sc ct</i>	..	..	..	..	..
<i>sc ct px</i>	..	..	..	..	..

were bred from normal females. Seven of these contained *ec* females, five only a few, comparable to the  $F_1$  result, but three about as many as there were males. In the latter cases the majority were not *px*, though Minute; when only a few *ec* females were present these were more frequently extreme *px*, namely, 8 *px*, 3 not *px*, though only  $\frac{1}{4}$  *px* flies ought to be found in all classes. In two more  $F_2$  in which  $F_1$  had not contained *ec*, 15 among 221 females were *ec*, M, extreme *px*. Again the *ec* females turned out to be sterile.

Where the *ec* deficiency is present it ought to show up also in such crossover tests which give crossover females. If, for example, the test is made with *sc ec ct*, few crossovers *sc-ec* (5.5 per cent) are expected, and not many double crossovers (0.8 per cent). An excess of the *ec* class among the females without an excess of the reciprocal class will indicate the *ec* deficiency. (The reciprocal class would contain one-half of the normal crossovers). Table 125 gives such a test for females, indicating also the *px* segregation, namely, (*px bl*  $\times$  *sc ec ct*)  $\times$  reciprocal, nos. 760-764.  $F_1$  did not show any *ec* deficiency; all  $F_2$  are sister broods. The table shows at once the large *ec* and *ec px* class, the reciprocal class being absent (only 0.8 per cent of both expected). In three cases the *sc ec* class is too high as compared with the reciprocal *ct* class, presumably by containing also *sc* individuals with *ec* deficiency.

We shall not discuss here the px numbers except to point out that one-half of the ec class is plexus. By way of contrast we add as table 126 a similar  $F_2$  in which no ec deficiency is involved, using as markers y w ec f.

Table 124 shows that in  $F_1$  the ec-deficient flies appear only in small numbers, far from one-half. As no females are missing and the deficient flies are perfectly viable, though sterile, there is no reason to assume that the mothers were heterozy-

TABLE 126  
(px bl  $\times$  y w ec f)  $\times$  (y w ec f  $\times$  px bl)

Class	No. 778		No. 776		No. 775		Total	
	♀	♂	♀	♂	♀	♂	♀	♂
+	41	29	24	5	29	25	94	59
+ px	10	6	7	1	8	4	25	11
y w ec f	25	32	16	18	18	22	59	72
y w ec f px	8	8	4	1	4	2	16	11
y	..	1	1	1	..	..	1	2
y px	..	1	..	..	..	..	..	1
w ec f	..	..	..	..	..	1	..	1
w ec f px	..	..	..	..	..	..	..	..
y w	..	..	..	..	..	..	..	..
y w px	..	..	..	..	..	..	..	..
ec f	1	..	..	..	..	..	1	..
ec f px	3	..	..	..	..	..	3	..
y w ec	25	8	22	2	19	5	66	15
y w ec px	2	1	3	1	4	3	9	5
f	24	15	19	14	22	20	65	49
f px	2	4	3	4	7	4	12	12
ec	..	..	..	..	..	1	..	1
ec px	..	..	1	..	..	..	1	..
y ec	..	..	..	..	1	..	1	..

The reduced male classes have already been discussed with the  $X^0$  crosses.

gous for the deficiency. Further, an  $F_1$  which had contained only one deficient female produced in  $F_2$ , from normal females, seven out of eight times  $F_2$  broods with ec-deficient daughters. In four cases the numbers were small, as in  $F_1$ , and in three cases as many daughters as there were sons showed the deficiency. To this ought to be added the two sister  $F_2$  broods (table 124) and the four sister  $F_2$  (table 125), all containing ec-deficient females. These facts indicate that as a rule the ec deficiency is covered by a duplication, which indicates that a small section of the first chromosome containing ec is translocated or transposed somewhere, and that this translocated piece may be removed by crossing over in  $F_1$  or  $F_2$ , or by recombination, or both.

Looking at the ratios encountered in tables 124–125, we find what looks like three groups, namely, (1) only a small percentage of ec flies, (2) about as many ec females as the number of males, (3) about one-eighth of ec females. The numbers are:

- 1) 8 broods with 625 ♀ 303 ♂ 9 ♀ Df = cr. 2 per cent
  - 2 a) 3 broods with 239 ♀ 162 ♂ 23 ♀ Df
  - 2, b) 4 broods with 191 ♀ (♂ not counted) 31 ♀ Df
  - 3, a) 2 broods with 109 ♀ 60 ♂ 42 ♀ Df = 2.6 ♀ : 1 Df
  - 3, b) 1 brood with 58 ♀ 2 ♂ 2 ♀ Df = 3.3 per cent Df
- } = cr. 8 ♀ : 1 Df (7 : 1 ♀)

This grouping also shows remarkable sex ratios, namely, about 2 : 1 in the first group, 1 : 6 in the second group, 2 : 5 in the third (a) group, and 30 : 1 in the third (b) group.

The ratio ♀ : ♀ Df = cr. 3 : 1 found in the third (a) group is explained if the non-deficient mother was heterozygous both for the ec-Df and the transposition into an autosome, and the father likewise (simplex for ec-Df). This requires lethality of the homozygous deficiency, but not of the homozygous transposition. If the mother was constituted as described, but the father was heterozygous for the autosomal transposition but did not contain the ec deficiency,  $\frac{1}{8}$  of the daughters should be deficient. The series of  $F_2$  from the same  $F_1$  in table 125 contains both types, as expected. The transposition is not expected to be in the second chromosome, since in all groups the deficiency recombines with px, i.e., both second chromosomes from px bl. We must therefore expect it to be in the third or fourth chromosome. In all groups assumed not to show a 7 : 1 segregation of the deficiency the segregation of px is as irregular as in the normal class and clearly independent of the deficiency (table 124). But in the group which we interpreted as a 7 : 1 segregation for the deficiency twice all deficiencies were px (15 individuals), twice half were px (16 : 15), and once 5 among 6. This can hardly be a chance result. The differential feature of this group is that the father does not contain the ec deficiency. It follows that in this case daughters heterozygous for ec-Df require the presence of two second chromosomes from px bl. The X chromosome introduced by the father must therefore contain something which is viable only or mostly in the presence of two X chromosomes from px bl stock, and the same thing must not be present in the X containing the deficiency. A translocation between 1 and 3 must somehow be linked with the ec-Df so that both are not present simultaneously in the same X. In view of the rarity of the deficiency and its sterility, no attempt at further analysis of this and some other problems presented by this Df was made.

The plexation in females with the ec-Df (uncompensated) was always extreme. There is a possibility (as we had assumed originally) that some connection with the abnormalities near the px and bs loci (see above) are responsible; but we did not succeed in establishing it. Possibly the high plexation is only an enhancing effect of the deficiency.

There remain still to be explained the  $F_1$  and  $F_2$  groups with only 2-3 per cent deficient females.  $F_2$  from such a cross (748-756, table 124) shows that many  $F_1$  flies were heterozygous for the deficiency. If the mother had been homozygous for the translocated ec locus, all  $F_1$  ought to have been normal. The small percentage of deficient females could thus not be explained. There is a possibility that these were survivors from an otherwise female lethal class, but we are at a loss to explain the production of such a class. The sterility of the deficient females did not encourage further search.

There remain the sex ratios, which are not different in principle from those found before, though the high ratios are more frequent. There is a male lethal ec-Df class involved which alone would not explain the ratios in the presence of the translocation of the ec locus into an autosome, though its addition to the other features producing male lethal classes might.

The segregation of  $F_2$  px females is also remarkable. In the six  $F_2$  where it was registered the segregation was as follows.



750.	78 :	12 = 6.5	d/PE = 2.89
751.	58 :	10 = 5.8	2.91
753.	73 :	8 = 9.1	4.58
754.	132 :	25 = 5.3	3.82
490.	107 :	30 = 3.6	1.17
491.	105 :	25 = 4.2	2.0

---

Total 553 : 110 = 5

This is far from a 3 : 1 ratio and is in almost all instances significant. Lethal female classes in the presence of both second chromosomes from px bl are possibly involved. There is again some relation between the translocation  $ec \rightarrow$  autosome and the not directly involved second chromosome, as we saw with respect to the linkage of plexus and the deficiency in some crosses, a relation difficult to visualize in detail.

Finally, one more peculiarity of these crosses should be mentioned. We remember that blistering is extremely rare in the  $F_2$  crosses with px bl, that with few exceptions, it appears only when both X chromosomes from px bl are recombined, and that the extremely rare crossover cases indicate a locus at the left end of the X as responsible for blistering, a locus which normally must be involved in a peculiar system of balance to explain the behavior of the px bl stock. Now in one of the  $F_2$ , no. 748, in which the females could contain only one of the px bl first chromosomes, a large number of blistered flies, all high-grade px, appeared among the px flies, and all the px- $ec$  flies were simultaneously blistered. This suggests a further relation of the rearrangement under discussion to the blistering effect, again very hard to understand or to analyze in detail.

#### 1. DOMINANCE AFFECTING SMALL TRANSLOCATIONS IN px bl

In our search for deficiencies at the suspected loci we found two more strange cases. In one cross (px bl  $\times$   $X^v$ )<sup>2</sup>, among 118 females, normal according to expectation, one female v was found. This was crossed to y v f males. One chromosome of the female was derived from  $X^v$  or a part of it after crossing over; the other was a pure px bl X chromosome. Therefore either the latter had mutated to v, or was deficient for v, or the recessive v had become dominant. In the first case, daughters as well as sons ought to be v (with the addition of eventual y and f in the females and grandmaternal as well as maternal crossover classes in the males). In the case of v deficiency, daughters ought to be as before and sons all v and  $\frac{1}{2}$  missing (also crossover types as before or  $\frac{1}{2}$  + if v deficiency were viable in the males). The actual result was:

24 ♀ +, 6 with abnormal abdomen	9 ♂ cv ct v m g f
12 ♀ v	2 ♂ cv ct v m g
24 ♀ v f 8 with abnormal abdomen	1 ♂ v
2 ♀ f	1 ♂ f
16 ♂ +	

This excludes both deficiency and mutation. As the main classes of the daughters were + and v f and the males had cv ct v m g f, the constitution of the mother was obviously  $\frac{+}{cv\ ct\ v\ m\ g\ f}$ . Half of the males were missing, and as + and cv ct, etc., were present in equal numbers in the males, the missing males were in both classes of X chromosomes, i.e., based upon an autosomal condition like deficiency or dupli-

cation by translocation. Approximately one-fourth of the + and v f females had abnormal abdomen. These might eventually represent the abnormal classes, viable in the female. The two crossover classes v and f are very unequal, but together they give a normal crossover percentage. If, however, a part of the v females were actually heterozygotes with change of dominance, they might have been based upon an autosomal recombination involving a translocation with an effect like Dubinin's cubitus interruptus. Unfortunately, this was not realized in time and no further tests with the v females were made. The facts are therefore only of importance as a case of rare survival of a combination which involves autosomes and the v region.

TABLE 127

Class	No. 1032	No. 1033	No. 1034	No. 1035	No. 1036	No. 1031 (mother px)	No. 1058
♀ y w.....	..	..	16 <sup>a</sup>	..	..	..	25
y .....	..	..	1	..	..	..	1
w.....	40	..	..	..	..	..	..
+.....	50	89	22 <sup>b</sup>	113	70 <sup>c</sup>	32 <sup>d</sup>	30
♂ y w.....	..	..	25 <sup>b</sup>	..	..	..	20
y .....	..	..	1	..	..	..	..
w.....	20 <sup>f</sup>	..	..	..	..	..	..
+.....	28 <sup>g</sup>	74	18 <sup>h</sup>	35	29 <sup>i</sup>	29 <sup>j</sup>	23

<sup>a</sup> 7 bobbed.<sup>b</sup> 10 bobbed.<sup>c</sup> 1 ♀ soft blist, 1 ♀ small eye.<sup>d</sup> 1/4 bobbed.<sup>e</sup> 8 bobbed.

Bobbed means phenotype only.

/ 1 ♂ notch.

<sup>f</sup> 2 ♂ spread.<sup>g</sup> 2 bobbed.<sup>h</sup> 11 ♂ dwarf, soft, part spread.<sup>i</sup> + bobbed.

The abnormal abdomen turned out to be somewhat genetic, as it reappeared in two grandchildren bred from abnormal abdomen, though never in later generations.

Another isolated case of importance was found for the left end of the X chromosome. Among numerous F<sub>1</sub> between px bl and different combinations involving the y and w loci the following result was obtained once:

No. 591: px bl × y w 71 ♀ 58 ♂ + 1 ♀ w not y

The exceptional w female was mated to a normal brother (which carried the px bl X) and produced:

No. 782: 127 ♀ + 45 ♂ +, 61 ♂ w

The white mother therefore was no homozygous mutant and had no deficiency for white, and she must have carried a suppressor for y. As the decisive feature must be present among the + females, seven of them were mated to y w stock males. The result is set forth in table 127.

The table shows twice 1/2 y w and 1/2 + females and males and a normal sex ratio. The mothers, daughters of the exceptional w females, were ordinary heterozygotes for y w and therefore the exceptional w females had had one y w chromosome. (There are also two crossover y females and one white male.) Four times only were normal females and males found in good-sized broods, two of which had a normal sex ratio and two a sex ratio of 3 : 1. The other X chromosome of the exceptional w female had therefore been normal and the constitution must have been just y w / + +, as if no w had been visible. But one of the broods contained 1/2 w 1/2 + in

both sexes and only half as many males as females. This result would be obtained if the mother were  $+/y\ w$  plus autosomal suppressor (duplication) for yellow. But this still does not explain why the  $F_1$  heterozygous grandmother, proved to have been  $+/y\ w$  without a  $w$ -Df, was phenotypically white, though this might have been a kind of Dubinin effect, dominance of  $w$  in the presence of a translocation with a break near  $w$  and containing the  $y$  locus. Therefore both white and red pairs from 1032 were bred. The results are shown in table 128.

White pairs produced only white daughters, but both  $yw$  and  $w$  sons, once in equal numbers, twice approximately 3  $y\ w$  : 1  $w$ , which agrees with the interpretation just derived. Red parents produced only normal daughters and half  $y\ w$  sons plus a few crossovers. The mother thus was heterozygous for  $y\ w$  and the

TABLE 128  
1190-92 = 1032<sup>a</sup> white, 1193 = 1032<sup>a</sup> +

Class	No. 1190		No. 1191		No. 1192		No. 1193	
	♀	♂	♀	♂	♀	♂	♀	♂
w .....	23 <sup>a</sup>	5 <sup>b</sup>	50 <sup>c</sup>	10 <sup>d</sup>	48 <sup>e</sup>	15 <sup>f</sup>	..	..
w px.....	9	5	..	..	..	..	..	..
y w .....	..	9	..	35	..	25	..	30
y w px.....	..	2	..	..	..	..	..	..
+.....	..	..	..	..	..	..	83 <sup>g</sup>	22 <sup>h</sup>
y .....	..	..	..	..	..	..	..	1
w .....	..	..	..	..	..	..	..	1

<sup>a</sup> 2 soft wings, 1 scalloped.  
<sup>b</sup> 2 ♂ spread, 3 soft.

<sup>c</sup> 7 soft.  
<sup>d</sup> 6 soft, 4 spread.

<sup>e</sup> 6 soft.  
<sup>f</sup> 5 soft.

<sup>g</sup> 16 soft, 1 spread.  
<sup>h</sup> 16 soft, 2 spread.

translocation producing the  $w$  dominance effect was absent in both parents. Again, a considerable number of abnormal individuals occurred.

The scalloped white ♀ of 1190 was crossed to a tester ♂ and produced half  $w$  half  $y\ w$  sons, whereas a sister ♀, also white, produced only white sons. In the offspring of 1036 (table 128), likewise, one ♀ among 69 which latter must have been  $1/2\ +/+$ ,  $1/2\ +/y\ w$ , was phenotypically white and scalloped (no. 1195). Unfortunately, she was sterile. We did not succeed in isolating the translocation, which thus was lost. Some more data on this case will be found in the section on mutation (p. 512). But it is remarkable that in  $F_3$  so many soft and spread individuals appeared, actually  $poi\ s$ , and that, in addition,  $F_2$  (table 127) contained individuals with short bristles (marked  $bb$ ), dwarfs, a notched male, and one soft blistered female. These types all failed to transmit their character to the offspring, and the soft blistered female was sterile. Obviously, together with the  $y$  suppression there had appeared by mutation  $poi\ s$  and, once,  $bran$ , as well as other types which might have been duplication effects in connection with the entire change. In the absence of other markers and criteria the analysis could not be pushed further.

#### m. *poi* AND *bran* ALLELES IN *px bl*

We saw that the most frequent mutants produced from *px bl* are the  $svr^{poi}$  alleles and the *bran* alleles, both of which appeared not only with the major upheavals in the stock affecting all *px bl* characters, but also individually on different occasions.

This necessitates a search for these mutants in the stock, maybe in heterozygous balanced condition. As a matter of fact, the stock was tested in the course of many years in all kinds of outcrosses which ought eventually to have revealed the presence of these mutants. But only *poi s* was ever found in the stock, though *poi* and *bran* alleles appeared repeatedly as new mutants. In some of the tables such occurrences were reported; others have not been mentioned. Thus, in a series of 17 backcrosses (*px bl* × triple) × triple, with 1,406 ♂♂, once 2 among 77 ♂, and another time 2 among 65 were pointed (*svr<sup>poi</sup>*). Similar cases were found so frequently that the pointed mutants were not tested except in the cases already reported in the section on pointed. The same applies to the rarer occurrence of *bran* and their combination types, some of which have been described.

At one time only, namely, in the winter of 1937–38, had the mutant allele *svr<sup>poi</sup>* spread in the stock and been frequently observed in crosses. Later, as has already been mentioned, very extensive special tests for *poi* and *bran* were made at different times with hundreds of individuals picked at random, and the results were always entirely negative. All the facts tend to show that *px bl* does not contain mutants as such at those loci, but that these mutants are rather frequently produced, may spread in the stock, and may disappear again.

#### II. THE SALIVARY-GLAND CHROMOSOMES OF *px bl*

Table 129 contains a check upon the salivary chromosomes of *px bl* made in the same way as for *poi* (see explanation, p. 345). An additional column contains a further check using 13 mass cultures with more than one gland per slide (one slide each). We see at once that the majority of the small rearrangements are the same as in pointed (see the discussion of *poi* and *poi h*). Ten additional changes have been noted. Plate 24, figure 1, shows a small deficiency of probably 3 bands in the X chromosome near the silver region, which was encountered only once. Plate 25, figure 3 shows another deficiency near the tip of X between the *pn* and *w* loci, which was encountered repeatedly. A little farther to the right another deficiency was found (pl. 24, fig. 2), which, according to the Bridges map, would be just to the left of *ec*. As an *ec* deficiency was found genetically, this was suspected to be its location. But Demeree localizes *ec* in 3 F1, 2. A test was excluded by the complete sterility of *ec*-deficient females, which can be distinguished only in the compound. In only one cross was the one-band deficiency no. 8 (pl. 25, fig. 8) found. This is exactly where vermilion is supposed to be located, and actually what might have been a *v* deficiency or a Dubinin effect involving *v* had been found.

In the second chromosome we have another of the tip deficiencies (no. 9), which have been sufficiently discussed for the pointed case. Near the right end of 2R the same disturbances in the *px* region as were described for *poi* are frequently encountered. In addition, a frequent occurrence is Df. no. 13, which is located just to the right of the assumed loci for *bs* and *ba* (pl. 24, fig. 8). Another single-band deficiency at (2L) 26 Cl is not contained in the table, as not found in this series. It is located at or near the *dp* locus.

A transposition in 3L and a deficiency near by have been listed with an interrogation mark because no sufficiently clear picture could be established, though there is no doubt that something is abnormal at these loci. The former is the D region; the latter is to the right of *h*. The only rather conspicuous rearrangement found

at all is no. 20, and it occurred only once (pl. 26, fig. 5). It is a nonreciprocal translocation from (3R)86D1-E11, i.e., a region near Stubble to (3L)62, i.e., near *ve*. This largest translocation of about seven bands is of importance because it suggests

TABLE 129

SALIVARIES OF *px bl*

(Number of bottles: *px bl* × Ore, 11; Ore × *px bl*, 5; *px bl homo*, 6. Number of slides, 110.)

No.	Rearrangement	Number of slides positive			Check 13 outside hetero- zygous slides, mass	Plate and fig.	Found in n crosses out of 35
		<i>px bl</i> × Ore (55 slides)	Ore × <i>px bl</i> (26 slides)	<i>px bl</i> (30 slides)			
1	Df2T(X)1A 1-4 <sup>a</sup> ...	10 ♀	12 ♀	...	1	...	Twice all slides of cross in 10 crosses
2	Df(X)1C 1-3.....	...	...	...	1	24/1	
3	Df(X)3A2.....	1 (1) ♀ <sup>b</sup>	(2) ♀	...	1	25/3	In 5 crosses
4	T(X)3C <sup>a</sup> .....	5	...	4 (2) ♀ <sup>b</sup>	..	25/2	Once all ♀ slides in 6 crosses
5	Df(X)3D 1-4.....	2 ♀	....	1 ♀	2	24/2	In 5 crosses
6	Df(X)4C9 and 4D1 <sup>a</sup>	...	....	...	7	25/4	In 7 crosses
7	Df(X)9A2, 3 <sup>a</sup> .....	4 ♀	2 ♀	...	..	25/1, 2	In 5 crosses
8	Df(X)9F 13.....	2 ♀	....	...	..	25/8	Only in 1 cross
9	Df(2L)21A.....	7 ♀ ♂	1 ♀	1 ♀	..	...	In 4 crosses
10	T(2L)24AB <sup>a</sup> .....	7 ♀ ♂	2 ♀ ♂	5 ♀ ♂	..	24/4	In 7 crosses
11	Df(2R)58D 5 <sup>a</sup> .....	...	...	6 ♀ ♂	..	26/1	In 4 crosses
12	Df(2R)58E <sup>a</sup> .....	21 ♀ ♂	6 ♀ ♂	1 ♂	2	24/7	In 13 crosses, twice all slides
13	Df(2R)60D 5, 6.....	11 ♀ ♂	....	3 ♀ ♂	5	24/8	In 13 crosses
14	Df(2R)60F 4, 5 <sup>a</sup> ....	22 ♀ ♂	6 ♀ ♂	...	2	27/4	In 13 crosses, once all slides
15	T(3L)61A <sup>a</sup> .....	7 ♀ ♂	1 ♀	...	1	....	In 4 crosses
16	Df(3L)61C 8 <sup>a</sup> .....	3 ♀ ♂	....	6 ♀	2	26/2	In 8 crosses
17	Tp? (3L)70BC, 71BC	12 ♀ ♂	6 ♀ ♂	1 ♀	2	....	In 11 crosses, once in all slides
18	Df(3L)67D?.....	...	3 ♀	...	..	....	In 1 cross only
19	Df(3R)84D 6, 7 <sup>a</sup> ....	16 ♀ ♂	6 ♀ ♂	25 ♀ ♂	..	26/4	In 15 crosses In 3 <i>px bl</i> and 1 Ore × <i>px bl</i> , all slides
20	T3R-3L.....	...	....	...	1	26/5	
21	Df(3R)86C 6-8 <sup>a</sup> ....	16 ♀ ♂	6 ♀ ♂	23 ♀ ♂	..	26/7	In 15 crosses In 3 <i>px bl</i> , 1 hetero- zygous, all slides
22	T(3R)100F <sup>a</sup> .....	15 ♀ ♂	....	...	2	....	In 7 crosses
23	T(4)102F.....	3 ♀ ♂	1 ♀	6 ♀	..	27/1	In 7 crosses

<sup>a</sup> Found also in *poi*; see tables 45, 46.

<sup>b</sup> No. in parentheses probable, not certain cases.

♀♂ found both in female and male larvae.

clearly that the smaller deficiencies encountered are also actually deficiencies by translocation to another place, which, as a rule, could not be found (if not, some of the apparent small deficiencies are actually insertions). When one or two bands in a little-explored region or in a region of faint bands are disturbed, a decision between deficiency and insertion is difficult in the absence of interchromosomal attraction for such small sections, or their breaking in the process of preparation.

Finally, in a number of cases a thick conspicuous band was found attached to the tip of the fourth chromosome (no. 23, pl. 27, fig. 1). Its meaning is obscure. One other, more frequent, occurrence is not contained in the table because of analytical difficulties. It looks like a 3-band deficiency just at the chromocenter of 2R, where analysis is rather unreliable. We spent much time analyzing what probably is the plexus locus within the bulb in 58E of the second chromosome. Here there is always an abnormality. But the paleness of the bands did not permit an unequivocal interpretation. In plate 28, figures 1-4, a few drawings are given, one of them from a homozygote which might be interpreted as a 1-band deficiency or a 1-band duplication. As this structure is always found, the px in px bl seems to be based upon a small rearrangement.

It is of interest to know how many simultaneous individual rearrangements could be found in the slides taken from the offspring of one-pair matings. Table 130 con-

TABLE 130  
NUMBER OF REARRANGEMENTS FOUND IN ONE-PAIR MATINGS

Class	No. crosses	Total no. rearrangements checked	Slides per cross	Maximum one cross	Minimum one cross	Average
px bl.....	22	23	5	10	1	5.9
svr <sup>poi</sup> .....	8	16	5	10	5	7.4
svr <sup>poi</sup> h.....	12	16	10	8	2	5.3

tains these counts, but it must be emphasized that the statistical picture is burdened with considerable errors because the counts relate to crosses in both directions and also to homozygous larvae. In the former case, the male cannot introduce some of the sex-linked deficiencies; in the latter, only heterozygous effects had been checked.

### 3. FURTHER FACTS ON MUTATION AT THE MAIN LOCI

#### a. THE STARTING POINT

The following general features of mutation in the px bl stock and its derivatives have been described:

1. The mutational phenomena occurred only once in a controlled-pair culture so that the entire offspring was affected. In this case, homozygous px and bs reverted to normal in one second chromosome. Simultaneously, heterozygous rudimentary (r) ♀ and one ♂ appeared; furthermore, svr<sup>poi</sup> and probably also bran, which, however, was discovered only in a later generation. In addition, the plus modifiers for px changed.

2. Exactly the same happened later in a stock bottle, though the seriation was unknown. Again, px and bs reverted to +, svr<sup>poi</sup> and bran appeared, but no rudimentary. In both cases also, other bran and svr alleles appeared (see nos. 1, 10 in table 151).

3. Afterward, the same mutants and alleles and reversions occurred occasionally either alone or in conjunction (see table 151). Thus bran and poi appeared repeatedly alone or together in inbred px bl or poi stocks or after outcrossing. Blistered reverted to plus with or without plexus; plexus reappeared where it had been absent; also svr<sup>poi</sup> reverted to plus.

4. A series of alleles of  $svr^{po1}$  and *bran* appeared after the manner of mutants. Both these mutants and reversions tended to appear after outcrossing. This applies also to other mutants to be analyzed below.

5. *Px bl* as well as pointed stocks were proved genetically to contain a number of small translocations and transpositions not including known loci.

6. Some of these could be found in the salivary chromosomes, others not. They contained only 1-3 bands.

7. Both stocks contained in the salivary chromosomes numerous small translocations and deficiencies which could not be proved genetically, since they probably did not contain known loci, the only exception being *ec-Df*.

8. A stock of the  $svr^{po1}$  allele of completely independent origin contained also a number of the same small rearrangements found in *px bl* and  $svr^{po1}$ .

9. The main mutants involved in the present discussion, *px*, *bs*, *svr*, and *bran*, cannot be called clear rearrangements; but all are associated sometimes or always with very small chromosomal changes in the respective regions.

We must now look into some of the details. If the hypothesis were proved that mutation is a chemical change produced in a gene molecule by chance or by unknown causes, all the facts would be side issues of no importance for the problem of mutation. We could be content to state that the *px bl* stock and its derivatives have a tendency to mutation and return mutation at the named loci, and the presentation of details beyond this statement would be superfluous. But the facts described, beginning with the change of an entire offspring of one pair from a closely inbred line which had been carefully watched, suggested that mutation is not a chance phenomenon localized in a gene molecule, but a phenomenon dependent upon conditions in the chromosomes themselves and somehow connected with minute rearrangements, though not necessarily being themselves visible rearrangements. Therefore all available details concerning the origin of the mutants may be of importance.

#### b. ADDITIONAL DETAILS OF THE FIRST CASE

I. General statement.—We begin with the original case which started this work, the details of which have been reported in the Introduction (see data and tables pp. 293 ff.). We found:

1. From a pair of inbred *px bl*, the ancestors of which had always bred in the manner described as typical for the stock, only 10 individual offspring were obtained, the parents still being alive and nothing being wrong otherwise. All females were blistered. Semisterility or inviability with what looked like a selection for blistering was the first abnormal phenomenon.

2. None of the offspring of these pure *px bl* flies were like the parents. Among the females,  $\frac{7}{8}$  were wild type. These turned out to be: (a) part homozygous +, i.e., in regard to *px*, *bs*, and the X chromosome, and a true-breeding wild type could be established; (b) a part was heterozygous for *px*, which turned out to be ordinary plexus; (c) some were heterozygous for rudimentary (*r*) and a normal X chromosome; (d) some had  $svr^{po1}$  in one and *r* in the other X chromosome; (e) the X chromosome with *r* could simultaneously contain  $svr^{po1}$ , though it is not certain that this was not the result of crossing over; (f) rudimentary could simultaneously be *px* +.

3. Wild-type males equaled one-half of the females in number.

4. Whereas *px* extracted from heterozygous *px* + bred true to ordinary *px*, i.e.,

without bs and blistering, the low-grade px flies, which were  $\frac{1}{8}$  of the females and about  $\frac{1}{6}$  of the males, with equality of the sexes, bred like the original px bl stock and obviously had the same constitution, i.e., also contained bs<sup>n</sup> and the condition for blistering in the first chromosome.

5. There was one rudimentary male.

Thus,  $\frac{1}{8}$  of the female offspring and  $\frac{1}{6}$  of the expected male offspring of pure px bl remained px bl. Seven-eighths of the daughters had lost bs, and had also lost px either in both second chromosomes (return mutation to +) or only in one of them. The locus px, freed of bs, was ordinary px. Simultaneously, both r and svr<sup>po1</sup> had appeared by mutation in the X chromosomes, probably in connection with the lethality of half of the males of this group alone, the px group having normal sexes. The single r ♂ might have been a survivor of the male lethal group. The female heterozygosity for svr<sup>po1</sup> and r in the presence of one ♂ r proves that these mutational steps must have already occurred in the grandparents and therefore were connected somehow with the lethality of most of the parental generation. We know that all these features recurred repeatedly, with the exception of the mutant rudimentary, which remained rare. We might thus say that the origin of rudimentary is not necessarily involved in the origin of pointed or the return mutations at the px and bs loci. Though the "mutations" and the ratios obtained clearly indicate some orderly event, it is difficult to reconstruct what had happened. But it is clear that the words "mutation and reverse mutation in a gene" do not have much meaning in such a case. The clear and orderly interrelation of the two mutational and the two return-mutational steps indicate that they are all the result of some major happening which I can only visualize as a simultaneous change of a mechanical nature in the chromosomes, like a pattern change of some kind, though it cannot be described as yet in terms of definite translocations, etc. If we look at known facts the only possible parallels are found among larger and easily verified translocations. When the Blond translocation originated, the phenotypes Bld, Bld(1)Df, and Bld(2)Df soon appeared together. In this case, a relatively large reciprocal translocation with incomplete lethal deficiency-duplication classes could be located from the breeding results. But a similar case involving minute parts of the chromosome, may be even below one salivary band, and thus allowing for homozygous viable duplications and deficiencies, not visible in the salivary slides, could hardly be proved genetically when no known loci are involved in the changes. It is this parallelism which led me to doubt that the conception of simple gene mutations could explain our case and all the consequent facts which fall in line, though in the present case it is impossible to tell in concrete terms what had happened.

II. Further analysis.—The further facts of this first case are in agreement with such conclusions, as will be seen in this more detailed study:

*The normal and low plexus segregants.*—We begin with a short report concerning wild type and standard px which were recovered in the case of "upheaval," as we might describe the happenings under scrutiny. As reported in the pedigrees (table 1), wild-type flies appeared in the first (and subsequent) upheaval in the px bl stock, namely, 136 ♀ 70 ♂ +, one-half of the males obviously being lethal. Three pairs of these flies gave (a) normal females and rudimentary males, (b) normal females and pointed and rudimentary males, and (c) 148 ♀ 70 ♂ +. Later generations bred from wild type either bred true or segregated into normals and pointed, or normals,



TABLE 131  
ORIGIN AND FURTHER GENERATIONS DERIVED FROM Low plexus out of px bl

Genera- tion	No.	Cross	+		poi		rud		low px		px bl		Remarks
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
P	4203 B	px bl.....	..	..	..	..	..	1	..	..	All	All	1 ♀ one side rud. wing and leglike structure Breeds true  px not poi   1 ♀ 3 ♂ px spread blist (see below) 3 ♀ px poi 9 ♂ soft spread px blist (see below) Breeds true 3 ♂ soft spread px bl
F <sub>1</sub>	4474 B	px bl × px bl.....	136	70	..	..	..	55	18	19	..	..	
F <sub>2</sub>	4612 B	4474 + × +.....	-151	..	..	65	..	..	..	..	..	..	
F <sub>3</sub>	4614 B	4474 px × px.....	..	..	..	..	..	..	..	..	All	All	
F <sub>3</sub>	4765 B	4612 + × +.....	199	..	..	67	..	49	2	..	..	..	
F <sub>3</sub>	4764 B	4612 + × rud.....	77	..	..	43	29	34	25	15	..	..	
F <sub>4</sub>	4977 B	4764 + × poi.....	73	35	..	19	..	41	43 <sup>a</sup>	29	..	..	
F <sub>4</sub>	4978 B	4765 + × rud.....	28	53	..	13	71	70	2?	..	..	..	
F <sub>4</sub>	4979 B	4765 + × +.....	48	46	..	41 <sup>b</sup>	..	..	..	..	..	..	
F <sub>4</sub>	4988 B	4764 px × px.....	..	..	..	42 <sup>c</sup>	..	24	71	42 <sup>d</sup>	..	..	
F <sub>5</sub>	5173 B	4977 px × px.....	..	..	..	4	..	14	132	33	..	..	
F <sub>5</sub>	5176 B	4988 px × px.....	..	..	..	..	..	..	All	All	..	..	
F <sub>5</sub>	5345 B	5173 px × px.....	..	..	..	..	..	..	155	135	..	6	

<sup>a</sup> 60 blist  
<sup>b</sup> 39 short bristles  
<sup>c</sup> 1/6 ♀ short bristles  
<sup>d</sup> 5 ♀ 7 ♂ px and poi

pointed, and rudimentary. But some of them also contained plexus, as was reported in the pedigree and is to be reported in detail below. However, the wild type, which bred true for many generations, later twice produced a few rudimentary males, as will be reported in the section on rudimentary. Otherwise it bred completely true and was finally discarded. Crossover tests had been made for all chromosomes of the wild-type flies. They showed completely normal behavior, normal reciprocal classes, and normal sex ratios. The crossover values were somewhat high, e.g., 28.2 instead of 17 for the interval facet-cut. To all purposes, this "reverted" mutant line was normal. Normal true-breeding flies were later produced again when the mass-mutation phenomenon was repeated in the stocks. It may be emphasized here that these normal flies had simultaneously lost px, the bs allele present in the px bl stock, and the conditions required for blistering.

As recorded in table 1, a low-grade plexus appeared together with +, poi, and r from px bl, as well as with the recovered typical px bl type in the first occurrence of mass mutation. Part of the pedigree of this low px line, which has bred true ever since, has already been given in table 1. More details (only those relating to low px) are contained in table 131 (which presents also a number of broods already reported with the origin of other types).

This table shows the following. Low px first appeared in about  $\frac{1}{8}$  of the females and in the same number of males, of which otherwise  $\frac{1}{2}$  were lethal. Thus the type is somehow released from px bl by a combination of an autosomal and a sex-linked condition, the latter being heterozygous for a male lethal condition in the mother, while all px males were free of this X chromosome. But the low-plexus individuals, when one pair was tested, produced a complete return to the px bl type, which bred true. From the normals in  $F_2$ , as well as from normal females and rudimentary males low px again segregated in  $F_3$ —in one case only 2 among 200, which looks as if release by crossing over were involved; in the other case, one-fourth of the not rudimentary females and males. Both parents, then, were heterozygous for this px. Whether it recombined with rudimentary was not checked (but judging from later work with rudimentary, which contains px, it did). In the presence of plexus no pointed was visible, though px and pointed were visibly combined in  $F_4$  and  $F_5$ ; but this was produced by a new allele, to be discussed later. From now on the low px bred true, though rudimentary was still present and a new type plexus spread soft blistered male was segregated (see below). A stock of the low px was established and bred true to type for many years (under the name Tpx). From the very beginning the sex ratios were most variable where the low px type was involved. In  $F_3$ , there were 131 ♀ 92 ♂; in  $F_4$ , 161 ♀ 124 ♂; in  $F_5$ , 75 ♀ 76 ♂; in  $F_6$ , 139 ♀ 56 ♂; in  $F_7$ , 155 ♀ 144 ♂.

The low px stock was crossed to different stocks containing standard px, producing 100 per cent px flies of very low grade, which bred true. We may therefore assume that here the original px from which px bl was derived had been restored, by removing whatever was responsible for the px bl type, i.e., bs, enhancers of plexation, and the locus for blistering in the X chromosome. When low px first appeared it was still partly heterozygous for poi, r, or both, as table 132 of outcrosses shows. The crosses 5669–5712B were made immediately after the px type had appeared, the others about ten generations later when px had been selected. The first crosses all show a very high sex ratio, ranging from 1.9 to 3.4. (One reciprocal cross,

5735, is normal.) This shows that at this moment the px ♀ contained X chromosomes which produced lethal combinations in sons. This condition was obviously present in both X chromosomes, as the crosses segregating poi and rud or + and rud show. Those with + and poi are not decisive because poi is sometimes not clear. Most probably, the lethal classes result from combinations of the X chromosome with autosomes, the mother having been heterozygous for the autosomal conditions. Obviously, translocations 1→autosome were involved of the type proven to be present in poi. The presence of soft spread males suggests that the allele  $svr^{poi\ bi}$  was also present. But it was not extracted from these crosses (see below).

TABLE 132  
OUTCROSSES OF low px SOON AFTER ITS ORIGIN

No.	Cross	+		poi ♂	rud ♂	n ♀ : 1 ♂	
		♀	♂				
5669 B	low px × 5 ple....	82	33	6	..	1 9	Some ♂ spread 1 ♂ abnormal abdomen; Bd and spread
5715 B	low px × ey.....	78	26	4	..	2 6	
5716 B	low px × ey.....	69	..	14	12	2 7	
5736 B	low px × X ple....	80	16	..	12	2 8	2 ♂ soft, 2 ♂ soft spread
5735 B	X ple × low px....	80	72	..	..	1 1	
5712 B	low px × triple...	54	16	..	..	3 4	
7673 B	low px × a sp.....	53	55	..	..	0 9	
7674 B	low px × a sp....	32	27	..	..	1 2	
7675 B	low px × a sp.....	82	61	..	..	1 3	
7676 B	low px × a sp.....	58	48	..	..	1 2	
7677 B	low px × a sp.....	41	29	..	..	1 4	

Actually, in the beginning, when pointed or rudimentary or both were still contained in this low px line all the features described for pointed X chromosomes were still present. The same tests for all the chromosomes were made as are described for pointed, but the results were the same: lethality of the X chromosome containing pointed in combinations with two foreign right ends of second chromosomes as well as the right half of the third, lethal crossover classes in the first pointing in addition to a transposition, modifiers for pointed, and abnormal sex ratios. In view of the complete parallelism no further data are presented, which would only duplicate those presented for pointed. After pointed and rudimentary had been selected out, the still abnormal sex ratios indicated that some of the special first chromosome conditions were still present. A standard translocation test (Patterson) gave completely normal results.

*Broad round (bran).*—In the offspring of the line under discussion the mutant bran also appeared and became visible in the fourth generation. It is not probable that it had been overlooked wherever poi was present, because the combination of both, called soft blistered, is too conspicuous. But it might have been overlooked in broods without poi. However, its first appearance does not favor such a view. If present before, it ought to have become visible in a simple Mendelian ratio, which it did not. The pedigree is shown in table 133.

TABLE 133  
PEDIGREE OF THE FIRST BRAN

No.	Cross	px		px poi		r		px etc. <sup>a</sup>		+		poi	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
4612 B	4474 <sup>3</sup> + × +	..	..	..	..	..	55	..	..	151	..	..	65
4764 B	4612 <sup>3</sup> + × r	25	15	..	..	49	32	..	..	76	..	..	33
4977 B	4764 <sup>3</sup> + × poi	43	29	..	..	..	41	..	..	73	35	45	19
	(6 bl)												
4988 B	4764 <sup>3</sup> px × px	62	35	9	7	..	24	1	3	..	..	..	..
5173 B	4977 <sup>3</sup> px × px	129	33	4	..	..	14	..	9	..	..	..	..
5345 B	5173 <sup>3</sup> px × px	155	141	..	..	..	..	..	3	..	..	..	..
5173 B	4988 <sup>3</sup> px poi × px etc. <sup>a</sup>	37	12	..	..	..	10	57	27	..	..	..	..
	5 bran												
5343 B	5173 <sup>3</sup> px bran × px Mass	195	194	..	..	..	16	3	3	..	..	..	..
		37 bl	12 bl	..	..	..	..	..	..	..	..	..	..
5350 B	5178 <sup>3</sup> px × px	36	33	..	..	..	..	..	10	..	..	..	..
		20 bl	8 bl	..	..	..	..	..	..	..	..	..	..
5351 B	5178 <sup>3</sup> px etc. × px etc.	107	127	..	..	..	..	47	56	..	..	..	..
5349 B	5178 <sup>3</sup> px bran × px etc.	141	135	..	..	..	..	43	49	..	..	..	..
5352 B	5178 <sup>3</sup> px etc. × +	10	6	..	..	13	14	34	44	..	..	..	..
5345 B	5173 <sup>3</sup> px × px Mass	155	135	..	..	..	..	..	9	..	..	..	..
5346 B	5173 <sup>3</sup> px × px etc. Mass	165	171	23	8	..	..	14	10	..	..	..	..

<sup>a</sup> 2 px etc. = type plexus soft spread blistered = px bran/px bran, svr poi bl.

The first two generations contained neither bran nor any of the bran poi combinations. But in the third generation bred from px parents (4988) it turned out that the parents had been heterozygous for bran =  $\frac{1}{4}$  of the females (probably  $\frac{7}{8}$ ) were px, and so were half of the males. Two-thirds of the other males were rudimentary. Furthermore, there were 9 ♀ 7 ♂ px poi, i.e., px flies which clearly showed pointed wings, which ordinarily is not the case when px is combined with  $svr^{poi}$ . Actually, in this case a new poi allele had originated (see below). In addition, 1 ♀ 3 ♂ of a new type appeared with wings soft-textured, plexus, blistered, and spread (held at right angles to the body). We reported that this type turned out to be the combination of pointed blistered with plexus. Poi blist (see p. 356) is the combination of bran/bran with the allele  $svr^{poi\ bl}$ . The  $svr$  allele alone produces a wing which usually looks like pointed but is frequently soft-textured, with a tendency to spreading. In the presence of bran the poi blist phenotype is seen, pointed but blistered wings with a fluctuation toward pointed and singed. The additional presence of px makes for the new type just described, soft spr px bl. Thus the few individuals homozygous for px bran in the second chromosome and the allele  $svr^{poi\ bl}$  in the first might be taken to indicate that bran had been present in some gametes of the px parents that one X chromosome of the mother had contained r (together with  $svr^{poi}$ ) and the other had contained in the majority of the gametes either +<sup>xxx</sup> or  $svr^{poi}$ , which latter does not show clearly with px. The presence of  $svr^{poi}$  is improbable because there were no soft blistered males and therefore the second X was +<sup>xxx</sup>. But in some of the gametes of both parents there must have arisen the allele  $svr^{poi\ bl}$ . Actually, the individuals registered as px poi also contained the poi bl allele and were heterozygous for bran. Thus about  $\frac{1}{8}$  of the segregants contained poi bl and most of them were heterozygous, with a few homozygous for bran.

From the same  $F_2$  (4764) a pair + × poi were bred (4977). Here +, poi, and px segregated with a sex ratio near 2 : 1, and in addition as many r ♂♂ as poi ♀♀. If part of the px individuals were invisibly poi, the segregation would be in half females +, half poi, i.e., the mother had been poi/r. The insufficient number of r males is frequently found and is based upon lower viability. In this case the properly computed sex ratio is normal. No homozygous bran is present. But a subsequent generation from px parents (5173) segregated px and r males with a majority of females px (px ♀ not containing poi, as the next generation shows). In addition, 4 females px poi appear, i.e., px/px (bran/+);  $svr^{poi\ bl}$ , and 9 ♂♂ soft spr px bl marked in the table "px etc.," i.e., px bran/px bran;  $svr^{poi\ bl}$ . Half of the females in the px and r classes are missing, which requires a lethal combination 1 → auto-some. Again, bran and the poi allele have made their appearance in what hardly looks like Mendelian ratios. A further generation bred from px flies (5345) contains 3 px etc. males among 144, i.e., again mutant-like appearance of bran and  $svr^{poi\ bl}$ .

The only px etc. female thus far obtained was sterile, but a female px poi from 4988 could be mated to a px etc. male. About  $\frac{1}{2}$  of the daughters were px etc., the others px; half the males were lethal, the rest about  $\frac{1}{2}$  px etc.,  $\frac{1}{8}$  r,  $\frac{1}{4}$  px. Aside from the males, this shows, as already assumed, that the mother px poi had been bran/+;  $svr^{poi\ bl}/svr^{poi\ bl}$ , r, and that the px etc. father was bran/bran;  $svr^{poi\ bl}$ .

In this brood (5178) were found, in addition, 5 ♀ px and bran. Their offspring (5348, 5349) showed that they had been only heterozygous for bran, i.e., bran had been dominant in these flies. As this locus did not continue to breed as a dominant,

we must assume that dominance modifiers had been present. But we remember from the section on *poi bl* that dominant alleles of *bran* had twice been segregated from this stock, so that the possibility cannot be denied that the *bran* found here was not the ordinary *bran* which was isolated later. However, here *px* was still associated with the near-by *bran*. We shall return later to this dominance phenomenon, as well as to the combination of *px* with *bran*.

The type *px* etc. bred true except for a small number of individuals which looked like *plexus*. But a stock which was established soon died out. We shall return later to the fact that *px* together with *bran* always produced unviability and sterility. The other crosses in table 133 agree with the foregoing analysis. In this case, then, *bran* had appeared as a mutant, not in conjunction with the already present *svr<sup>poi</sup>*, but only with the new allele *svr<sup>poi bl</sup>*. We shall find that same association again.

At the time of these happenings a number of checks were made in order to throw light upon the first appearance of the combination of the two new mutants. Standard translocation checks were made. These were completely negative, showing that *px* etc., did not produce lethal hyper- or hypoploid combinations, not even clearly sublethal ones. The salivary chromosomes were checked, at that time only for translocations of a size easily discovered, such as *Bld*. The result was negative. First and second chromosomes of comparable phenotypes were both checked for deficiencies in the regions involved. The result was again negative. We also tried to establish a stock over *y*. *F*<sub>1</sub> showed normal ♀♀ and ♂♂ with soft, somewhat spread, wings which were found later to be typical of the *poi bl* allele. In *F*<sub>2</sub>, *bran* segregated in the females and *px*, etc., in the males, and breeding from *y*, *px bran/px bran* × *px* etc. ♂♂ a stock was established which likewise died out after two generations.

When we tested the X chromosome in the backcross (*px* etc. × *xple*) × *xple*, no *px* etc. males were obtained as expected (no *bran/bran*). Otherwise the cross showed clearly the presence of a transposition from the left end to the right end of the first chromosome of the same type as was described above for *poi*. Plus and *xple* classes had normal sex ratios. All crossover classes with the right end present but the left end up to vermilion replaced were normal. But the classes containing the *sc-ec* region from the *px* etc. chromosome but with the right end replaced had extremely high sex ratios in favor of the females. For single crossovers the numbers of reciprocal classes of these categories were 39 ♀ 35 ♂ against 41 ♀ 9 ♂ (which is highly significant statistically). If both the left and the right ends are replaced, or only the intermediate part, sex ratios are normal. We do not know whether this transposition is in any way connected with the appearance of the two mutants.

A check of the second chromosome, also made immediately after the appearance of the mutants, showed a disturbance in the *arc* region (where *bran* is located). This results in sublethality of males in which the *arc* region from *px* etc. is replaced, i.e., a/a crossover males which still have the left part of the original second chromosome including the *vg* region. Thus all crossover classes which have in one second chromosome both the *vg* and a region from *px* etc. are normal, as are those in which both regions are replaced. But when the *vg* region from *px* etc. is present and the *a* region is replaced (even if the *sp* end is present), very few males survive. The numbers for the first group were 21 ♀ 32 ♂; the second group, 35 ♀ 6 ♂ (statistically highly significant). All 6 ♂ of the latter group are in the *a sp* class, none in the double crossover *a* class without *sp*, which puts the disturbance to the left of *arc*.

Unfortunately, circumstances forced me to break off at this point and prevented me from determining more closely what happened to this region.

*Return mutation of plexus.*—Once more, again only a few generations after the first upheaval, the type px etc. = bran px,  $svr^{poi\ bl}$  homozygous was segregated in connection with happenings at the px locus, this time not reverse mutation to normal, but the reappearance of the lost mutant px. This remarkable case happened in the following way. The two alleles  $svr^{poi}$  and  $svr^{poi\ h}$ , both from inbred stock but completely independent in origin (see p. 299), were frequently crossed and gave a pointed compound. But once, strangely enough, plexus flies appeared in some  $F_2$  and backcrosses. Poi was derived from px bl but never had shown any plexation.

TABLE 134  
ORIGIN OF px FROM poi × poi h

No.	Cross	pointed		px		px bl		Remarks
		♀	♂	♀	♂	♀	♂	
5833 B	(poi × poi h) <sup>2</sup> 5681 <sup>2</sup> ...	44	33	13	14	1	..	1 ♀ 4 ♂ soft blist spread poi not px
5834 B	(poi × poi h) <sup>2</sup> 5681 <sup>2</sup> ....	73	79	23	16	1	.	
5877 B	(poi h × poi) <sup>2</sup> 5743 <sup>2</sup> ....	All	All	.	..	..	..	
5878 B	(poi h × poi) <sup>2</sup> 5743 <sup>2</sup> ....	All	All	..	..	..	..	
5839 B	(poi × poi h) 5681 × poi.	50	24	2	1	.	..	
5840 B	(poi × poi h 5681) × poi h	77	45	..	..	..	..	
5887—								
88 B	poi h × (poi h × poi) 5743	186	142	..	..	..	..	
5891 B	poi × (poi h × poi) 5743.	40	35	..	..	..	..	
5889 B	(poi h × poi) 5743 × poi h	128	77	..	..	..	..	
5890 B	(poi h × poi) 5743 × poi	79	65	..	..	..	..	

It had been bred under control for three generations of brother-sister matings, and afterward in stock bottles. Stocks were controlled and no px fly was ever found, nor an extra vein of the type indicative of heterozygous plexus. The same applies to pointed h, which had bred true for many more generations and was originally not derived from px. But in later years, i.e., more than five years after the crosses to be reported were made, one or two plexus flies occasionally were found in  $svr^{poi}$  bottles, certainly not more than one in thousands (breeding true as px after crossing to standard px). In innumerable one-pair crosses or in  $F_2$  of outcrosses with poi a px fly was never found; this includes one very large series of poi × poi h and  $F_2$  made just for this purpose. Table 134 contains all the  $F_2$  results of 1935 in which px reappeared. Plexus flies appeared in two  $F_2$  with poi mothers both derived from the same grandmother, and a few in a backcross of the same  $F_1$  to a poi male, again from the same grandmother. None of the other combinations contained px flies, but one backcross of a male, again from the normal reciprocal  $F_1$  no. 5743, to a poi female produced soft blistered spread flies, which, as we shall see, at once were producers of px flies, i.e., heterozygous px. (We recall that px does not clearly show pointed, and also that the pale body color is not reliable. This applies to the alleles poi and poi h, not to poi bl and poi si and others which are clearly visible with px. Later tests of the extracted px confirmed the absence of pointed.)

The sex ratios are remarkable. They are normal in the  $F_2$  producing plexus, but not normal in the backcrosses, except the one with the soft blistered spread flies: twice the ratio nears 2 : 1 (257 ♀ : 147 ♂), twice it nears 4 : 3, namely, 264 ♀ : 207 ♂. The  $F_2$  containing px flies has 229 pointed : 68 px, which is near a 3 : 1 ratio, though neither of the grandparents had shown plexus and none of their ancestors for many generations, including brother-sister matings over more generations. Nevertheless, the cross showed that one of the grandparents had contained plexus in a heterozygous condition.

The first idea to suggest itself is that the poi female, mother of  $F_1$  no. 5681, carried simply heterozygous px from the ancestral px bl line. This can easily be disproved :

1. When the poi stock was established, it was first carried for some generations in brother-sister matings, later in mass culture. Segregating flies would have been noted, though it cannot be denied that by chance all matings were  $+/+ \times px/+$ .

2. If this explanation were true, one-half of the  $F_1$  flies would have been  $px/+$ , one-half  $+/+$ . Three  $F_2$  and  $RF_2$  which could show such heterozygosity all contained px. (This would not be a proof in itself.) But there was never an indication of the heterozygous px effect, for which the flies were closely watched and which otherwise was never absent.

3. The backcross 5839 which produced a few px flies could do this easily if a few sperm cells of the father from poi stock contained px.

4. We have met with other cases of unexpected reappearance of px in a few individuals, where a simple former heterozygosity is excluded. We cannot, however, exclude the presence of a px duplication in pointed, though it was never found in the many test crosses with second chromosome markers containing px.

5. Numerous crosses for the analysis of the pointed alleles were made simultaneously, but no px segregation occurred; the heterozygous px effect was never observed.

6. Unequivocal proof that something else had happened is the absence of pointed from part of the extracted plexus flies, though both parents had been homozygous pointed (or simplex). Details will be given at once. A stock which was established from selected plexus flies was free from poi.

7. There is the cross 5891 with a father from 5743, which latter did not produce px in whatever crosses. With a poi mother a few soft spread blistered flies (not px!) were produced which turned out to be heterozygous for px and homozygous for poi bl. It follows that px is either a return mutation occurring more or less regularly in poi, or that it is carried in a heterozygous condition covered by a closely linked duplication which only rarely is set free by crossing over. Thus it might or might not be relevant that px was set free after crossing the two poi alleles. But it is certainly important that *svr*<sup>po</sup> had simultaneously mutated back to +, i.e., reversed the original happenings, and that now bran and poi made their appearance.

Table 135 shows the  $F_2$  results of breeding from the  $F_2$  5834 and the backcross 5891. (In addition, the soft spread blistered males and px females and males were tested for deficiencies in the first and second chromosomes, without results.) The first two items of this table show a remarkable result. The plexus flies bred true to a plexus just as extreme as in the px bl stock, and furthermore, half of them, both males and females, were blistered; but half of the blistered males were missing, which is the case in both crosses. (In the standard px stock, blistered males are very



rare, within a normal sex ratio.) Thus we find the male lethal class, so frequent in outcrosses with poi, and simultaneously the new appearance of blistering in both sexes, male lethality being confined to the blistered class (106 ♀ px 111 ♀ px bl. 87 ♂ px 46 ♂ px bl), a condition which we already know to be typical for bs<sup>pp</sup> in conjunction with px bl. As pointed certainly did not contain bs, which was tested innumerable times and could not have been overlooked, this means that bs had reappeared in the form of the allele bs<sup>pp</sup> together with near-by px.

A further generation bred from not blistered flies gave 70 ♀ 35 ♂ all extreme plexus and blistered, one-half of the males missing, as was previously the case with px bl males (table 135). A line was extracted which has ever since bred true for strong plexus but varies in regard to blistering. A test made after five years showed only a few blistered flies, but after selecting had been done for some time most flies

TABLE 135  
SOME F<sub>2</sub> FROM F<sub>2</sub> IN TABLE 134

No.	Parents	px		px bl		poi		soft px ± blist ± spread		poi blist	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
6046 B	5834 px... ..	73	64	58	26	..	..	..	..	..	..
6047 B	5834 ♀ px bl ♂ px .....	33	23	53	20	..	..	..	..	..	..
6048 B	5834 pointed.....	..	..	..	..	73	39	..	..	..	..
6063 B	5891 ♀ ♂ soft spread bl.....	..	2	..	..	49	48	16	12	5	2

were blistered. We know from an earlier section that the allele bs<sup>pp</sup> was present, which produces blistering in both sexes and high plexation, and this constitution was confirmed in the present case.

To return to the other crosses of table 135, we see that the pointed flies from the same F<sub>2</sub> 5834 bred true to pointed. One count, however, showed 1/2 of the males missing, a sex-linked lethal obviously introduced from the grandparents. The strangest result was obtained from breeding the soft spread blistered individuals (not px) segregated from backcross 5891. About three-fourths of the offspring were pointed. The rest consisted of a majority of soft blistered plexus and partly spread individuals, indicating the presence of bran and poi bl; some females and males were pointed blistered without px; and two males were only plexus. Both parents had been heterozygous for px and homozygous for a poi allele (poi bl), px having been absent in two former generations. In this case bran had again obviously been somewhat dominant, as the blistering indicates, the other characters being produced by the poi allele. Bran and px, then, had arisen in the same second chromosome. (The 2 px ♂♂ were minus variants of the same class.) Also, there were 5 ♀ 7 ♂ pointed blistered. They turned out to be heterozygous for px and homozygous for bran and svr<sup>poi bl</sup>; actually, the stock poi blist was derived from these individuals after segregating out px. As the parents had been bran px/+, svr<sup>poi bl</sup>, the production of poi blist = bran px/bran, svr<sup>poi bl</sup> required either crossing over bran-px, which is not probable with so high a percentage, or a loss of px. Unfortunately, the 2 males px were not tested for bran, since at that time the constitution of poi bl had not yet been cleared up.

Thus we see in this line (1) the reappearance of px after crossing the two poi alleles, one of which was derived from px bl; (2) the reappearance of bs as bs<sup>pp</sup>; (3) the mutation to bran; (4) the mutation to svr<sup>poi bl</sup> from the svr<sup>poi</sup>; (5) the reversal of svr<sup>poi</sup> to normal; (6) male lethal classes involving translocations and apparent transpositions within both first and second chromosomes, which might have been present already and need not necessarily have been involved in the happenings. It might be added that the new pointed blistered was immediately checked for translocations with the Patterson test, with negative results except for the already reported suppression of eyeless. The same negative results were obtained for px bs<sup>pp</sup> which had arisen here.

A new feature of these happenings (no. 5) already mentioned was the disappearance of poi in part of the px flies. This first became apparent (px with ordinary poi

TABLE 136  
F<sub>1</sub> CROSSES OF NEW px bl (SEE TABLE 135)

No.	Cross	♀ hetero- px	a ?	♂		+	poi blist	Remarks
				poi	px bl			
6186 B	6046 px bl × b pr vg a sp	109	..	27	1	..	..	} px hetero effect more pronounced than usual hetero-e <sup>a</sup> effect
6190 B	Reciprocal.....	24	3	..	..	21	..	
6212 B	6047 px bl × triple....	68	..	7	..	..	1	
6231 B	6047 px bl × 5 ple.....	44	1	..	..	4	4	
6232 B	6047 px bl × X ple.....	137	30	40	..	..	1	
6210 B	6047 <sup>a</sup> px.....	119	..	7	..	13	..	

and especially if blistered is difficult to recognize clearly) when the new px bl type (with bs<sup>pp</sup>) was checked for crossover in different chromosomes immediately after its appearance. The F<sub>1</sub> crosses are contained in table 136. The mothers px or px bl twice produced both pointed and normal sons, the others only pointed sons. Six males were pointed blistered, i.e., bran + poi bl, again with some dominance of bran. This shows again the presence of the new bran and the allele svr<sup>poi bl</sup> in a few gametes of the new px bs<sup>pp</sup> ♀. The heterozygous a and e effects occurred just as in the ancestral pointed line. The heterozygous plexus effect was more pronounced than usual, probably owing to the bs<sup>pp</sup> allele. Most conspicuous are the sex ratios, both in the presence of only poi or of poi and + males. They were 3.9 : 1, 8.5 : 1, 5.6 : 1, 4 : 1, 6 : 1. Obviously, lethal recombinations of X chromosomes and autosomes are found, which means that the mother was heterozygous for translocations from X to autosomes producing male X-deficient classes. Again, we suspect that these lethal recombinations had some relation to the changes under discussion. (The stock which was selected from px bl gave normal sex ratios when tested after many generations. The absence of lethal classes in translocation tests must be explained as in former cases.) We cannot help assuming that the few poi blist and one px bl ♂ "mutant" were somehow survivors of otherwise missing classes.

Both males + and poi were tested in backcrosses; they were px/+, as expected, and otherwise gave normal results. The females, too, were tested for the second chromosome in quintuple backcrosses. They might have been poi/+ or +/+ for the X chromosome. Actually, there was no suppression of speck. Only 19 among 110 ♂

TABLE 137  
OFFSPRING OF FLIES FROM THE CHANGED 369 STOCK

No.	Parents	+		poi		soft bl		px (bl)		px etc. <sup>a</sup>		poi sing		Remarks
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
3969	♀ ♂ poi sing.....	6	10	5	10	75	30	..	..	..	11	..	..	+ and poi hetero-px poi not clear
3970	♀ ♂ +.....	..	..	9	19	22	6	3	4	..	1	..	..	
4481	♀ ♂ +.....	44	54	..	..	..	..	13	22	..	9 <sup>b</sup>	..	..	
4482	♀ ♂ +.....	50	38	12	18	..	..	9	11	..	..	11	4	15 ♀ 11 ♂ px poi blist
4491	♀ ♂ +.....	64	54	20	19	..	..	15	5	..	3	21	3	Part + hetero-px
4492	♀ ♂ +.....	94	65	..	26	..	..	25	21	..	3	..	..	

<sup>a</sup> px etc. = type plexus soft spread blistered = px bran/px bran, svrpoi bl.  
<sup>b</sup> 8 without px. + ♂ poi ?

which could show it were registered as pointed, all in the normal class. The other abnormalities were: (1) In the noncrossover quintuple class  $\frac{1}{2}$  ♂ were missing (69 : 39). These might be the expected poi ♂. (2) In the large crossover classes containing the a locus, half of the ♂♂ were missing (88 ♀ : 40 ♂). (3) In the + class (with 19 ♂ registered as poi) the sex ratio was 107 : 74 = 1.4. If the missing males are added to the 19 poi ♂, ♂ equality of + and poi is seen (55 : 52). Thus it is possible that  $\frac{2}{3}$  poi ♂ were lethal in the + class, and all in the quintuple classes and those containing arc. The arc region is again involved, in this case being needed for survival of pointed. The complete sex ratio was 300 : 193 = 1.7. (Crossover values did not furnish further information, as was always the case in the present study, probably because only minute rearrangements were involved.) Thus, we see again a complicated relation of the arc region with the X chromosome.

### C. THE SECOND UPHEAVAL

In the Introduction, we reported upon another "upheaval" in the px bl line which likewise resulted in the appearance of bran and poi alleles and their typical recombinations, together with the disappearance—so-called return mutation—of px and bs. The line in question had already been extracted for the bs<sup>sp</sup> allele, which had appeared under the following circumstances. In a bottle of px bl, 1 ♀ 3 ♂ were found notched, otherwise typical px bl. The males were tested in F<sub>1</sub> and F<sub>2</sub> with y and notches did not appear again. One notched pair gave only 8 ♀ ♂ offspring, not notched but blistered. A subsequent generation contained only extremely plexus blistered individuals, females as well as males, and this new condition bred true, though not all individuals were blistered. The stock remained so for years and was kept as no. 369. It turned out that this was actually px bl with the higher allele bs<sup>sp</sup> present (see p. 433). There was no more notching, but it might be more than a coincidence that notching appears in males with bran opposite an a px deficiency, which suggests that the beginning had, again, been something like a deficiency by translocation.

After three and one-half years in which nothing happened in this stock, one of the bottles again contained, in addition to the type of the stock, wild type, low plexus, pointed, pointed singed, and soft spread plexus blistered flies. Thus, not only had px and bs<sup>sp</sup> disappeared again, but pointed or other alleles of svr<sup>po1</sup> and also bran had appeared in a part of the flies. Table 137 gives the results of breeding from 5 pairs out of that bottle. The following is an attempt to analyze these happenings.

The parents were wild type four times. All turned out to be heterozygous for px, which segregated altogether as 56 ♀ 60 ♂ among 334 ♀ 312 ♂, i.e., a ratio of 4-5 : 1. Once (4481) the parents contained neither poi nor bran, and segregated px in nearly a 3 : 1 ratio, which means that no lethal or poorly stable combination with the second chromosome occurred. In this case a relatively large number of "px etc." males segregated, of which, however, only 1 was px, 8 being only soft blistered spread (but not of the "soft blistered" type containing poi with bran), i.e., they contained bran and poi blist. But there is a possibility that the mother was heterozygous for poi, since part of the males might have been poi with poor expressivity. In no. 3970, only 1 px etc. ♂ segregated among 30 ♂. Plexus segregated in a very high ratio. More than half ♀♀ and few ♂♂ were soft blistered. This normally requires that one of the parents be homozygous, the other heterozygous for bran, and both poi; neither of

which had been visibly the case. However, as the offspring did not permit clear distinction between + and poi, some of the modifiers of the phenotype might have been involved. But even then one of the parents ought to have been soft blist, not normal. In 4491 the ratio + : px is again very high, 7 : 1 for ♀♀ and 9 : 1 for ♂♂. About half of the females are pointed but only one-fourth of the males, the other fourth being missing; again, the father must have been poi without showing it, and the mother poi/+. We shall see in table 138 that the extracted pointed singed flies bred true. They were extracted simultaneously from a sister brood, 4482 (p. 484), and shown to contain the new alleles bran<sup>3</sup> and svr<sup>po1</sup>, which together produced the poi sing type (varying into poi), and in the presence of px the type plexus pointed blistered. Thus, at least the alleles bran, bran<sup>3</sup>, poi bl, and poi si appeared after the return mutation of px and bs<sup>pp</sup> in this stock, and eventually also poi.<sup>18</sup>

Still more complicated are the offspring of 3969, with both parents poi sing from the stock under scrutiny. It is impossible that this poi sing was the one just mentioned (bran<sup>3</sup> + poi si), which latter breeds true. Seven-eighths of the daughters were soft blistered, a result which is only possible if both parents have some compound of bran alleles and one is heterozygous for a compound of poi alleles one of which does give ordinary poi (+ flies being probably poi) with the one-fourth of the segregating homozygous bran allele. As bran<sup>3</sup> has no visible action alone, nor with poi (only with poi si), the parents might have been bran<sup>3</sup>/bran, which would segregate 1/4 bran<sup>3</sup>/bran<sup>3</sup>. As 7/8 ♀ were soft blistered, bran/bran and bran<sup>3</sup>/bran must give soft blistered with both possible poi compounds; but as the parents were soft blistered, these combinations for poi must not have existed in the parents. This is only possible if the mother had one poi compound and the father a different poi allele. As part of the sons (probably one-half, the other half being lost owing to the poor viability of the type) were of the type px etc., which contains bran and poi bl, the maternal compound could have been poi si/poi bl. Thus the cross had been

$$\text{bran/bran}^3, \text{poi si/poi bl} \times \text{bran/bran}^3, \text{poi?}$$

Six of eight recombinations have bran/bran or bran/bran<sup>3</sup> with, in the daughters, either poi si/poi? or poi bl/poi?. As all are soft blistered, poi? can be neither poi si nor poi bl (see table of phenotypes). One-fourth of the daughters are bran<sup>3</sup>/bran<sup>3</sup>, again with both poi combinations. As one is actually soft blist, the other poi (incl. plus), poi bl/poi? obviously produced also soft blistered with bran<sup>3</sup>, but poi si/poi? only pointed. In the sons the results would agree with this interpretation, bran<sup>3</sup>/bran<sup>3</sup>; poi si being the 1/8 poi and plus sons, the rest being 1/2 soft blistered with bran/bran etc. and poi si and 1/2 (poorly viable) soft spread blist with poi bl. But there is one difficulty left: all poi blist soft spread males are plexus and no females px are found. The parents must have been px/+, and px/px appeared only in males—moreover, only in males with poi bl. This cannot be visualized. The only visible explanation is that only one of the parents was px/+ and that px was more dominant than usual in the presence of poi bl. As the stock originally contained bs<sup>pp</sup>, which increases the heterozygous effect, this is rather probable. At the time of this experiment the different bran and poi alleles were just being isolated, so that the decisive tests could not be made. But a comparison with the simultaneous

<sup>18</sup> We add that a contamination is excluded because bran<sup>3</sup>, poi si, and poi dish had not existed before, while bran was present in combination with near-by px. The same is true for bran<sup>4</sup>. Furthermore, no pure bran stock was kept at that time.

analysis of the changed stock 369 shows that the alleles needed for the foregoing interpretation had actually been produced in this stock.

Still another complication was found which could only be understood after the different bran and poi alleles had been analyzed. When analyzing the poi sing types from 369 which were combinations of bran<sup>a</sup> and poi si, bran<sup>3</sup> alone having no visible effect, we found a low plexus poi sing male which had no pointed wings. It was crossed to Oregon, and in a mass F<sub>2</sub>, normal, plexus, bran, and px bran females and males, and pointed and pointed singed males segregated. The original father thus had been heterozygous for plexus, which had shown more dominance than usual, but also for bran, which is located next to px, i.e., px bran/bran, and the mass F<sub>2</sub> permitted the combinations of px bran and bran homozygous, i.e., px bran, px bran, bran/bran, and px bran/bran. But there were also many px without bran, too many to be crossovers; furthermore, no soft blistered appeared. In addition, poi and poi sing males appeared in a number far below one-half (12 poi, 1 poi sing among 85 males). This looks as if poi si had appeared as a mutant in a part of the F<sub>1</sub> males, which would mean in one-fourth of the gametes with the X from the original father.

All segregants of this F<sub>2</sub> were tested. Bran inbred or crossed with a bran stock was sterile in four cases. Bran px was twice sterile among four bottles in mass culture; twice it bred true, but in small numbers. A stock was established but died out, showing that the sterility of soft blist px, discussed above, was due to the bran px combination, as was found before. Since we had found a one-band deficiency in what probably is the bran locus in at least one allele, and also a one-band deficiency at or near the px locus, this might furnish the explanation. Once, bran px from one F<sub>2</sub> was tested with bran stock. The F<sub>1</sub> was normal (not bran!) and segregated in the next generation into +, bran, px, and px bran, showing that the tested bran px ♂ had either been heterozygous bran px/px with considerable dominance of bran or homozygous for a bran allele, which does not give broad round together with bran. The same phenomenon was observed when + × bran px was bred from one F<sub>2</sub>. It segregated into ½ + and ½ px, showing that + had been heterozygous for px. Moreover, all px flies showed transition from + to bran, including both extremes. The same was true in F<sub>3</sub> made from bran px × poi, in which poi proved to be also px/+. The F<sub>2</sub> ♂ poi singed was mated to a sister bran. The offspring of half bran, half normal showed this male to have been bran/+, whereas all other poi males which were tested did not contain bran. Thus poi had originated by mutation, as was already suspected, in gametes not containing bran, though px could be present. But in one gamete poi had originated in an F<sub>1</sub> mother heterozygous for bran. Actually the poi mutant reoccurred in F<sub>2</sub>: one pair + × bran produced 48 ♀ 31 ♂ + 2 ♂ poi!

It turned out that the poi which had appeared in this group was the allele *svr<sup>poi bl</sup>*, since typical poi blist flies segregated in F<sub>4</sub> from bran px × + in F<sub>3</sub> (together with bran and bran px) and also from F<sub>3</sub> bran × bran out of bran × poi sing in F<sub>2</sub>, as well as from normals of F<sub>3</sub> out of the same F<sub>2</sub>. As poi blist flies tend to show a phenotypic variation from poi over poi sing to poi blist, the possibility exists that some of the poi flies of F<sub>2</sub> were actually poi blist, i.e., bran/bran, *svr<sup>poi bl</sup>*. Tests, however, did not show them to be homozygous for bran, so that the former discussion of those poi males is not impaired except that the allele in question is poi bl, which is not always distinguishable in the absence of bran.

TABLE 138  
EXTRACTION OF bsp<sup>sp</sup>, brun<sup>3</sup>, AND poi si FROM THE 369 STOCK

No.	Cross	px extreme		px extreme blist		px poi blist		Remarks
		♀	♂	♀	♂	♀	♂	
4490	Extreme px bl from stock.....	50	48	25	22	..	..	Extra hair and bristles; 1 ♀ 1 ♂ low px 9 ♀ 9 ♂ px poi not blist; px poi are hairy 44 ♀ 53 ♂ +; px is not extreme; short bristles in all classes Breeds true Breeds true
4662	4481 <sup>1</sup> px bl × px (see table 7)...	..	67	63	25	..	..	
4681	4490 <sup>2</sup> px bl.....	34	40	..	..	59	12	
4682	4492 <sup>3</sup> + × px etc. (see table 7).	27	35	11	1	..	..	
4912 ff	4662 <sup>2</sup> diff. comb.....	..	Some	All	Most	..	..	Breeds true Breeds true
4956	4681 <sup>1</sup> px, or px poi, or px poi bl.	..	..	..	..	All ±	All ±	

TAB  
SELECTION IN  
x bl 3

No.		+		poi		px poi bl		px		poi sing		Remarks
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
4482 F <sub>1</sub>	♀ ♂ + from stock. ....	50	38	13	18	15	..	9	11	11	4	3 ♂ px etc.
4491 F <sub>1</sub>	♀ ♂ + from stock. ....	64	54	20	19	..	..	15	5	21	3	20 ♀ 11 ♂ px poi sing
4663 F <sub>2</sub>	4482 <sup>2</sup> poi sing. ....	..	..	22	36	..	..	2	6	48	20	Extra venation
4664 F <sub>2</sub>	4482 <sup>2</sup> poi sing. ....	..	..	36	40	..	..	..	..	34	12	30 ♀ 4 ♂ poi sing all px; px ♂ poi
4665 F <sub>2</sub>	4482 <sup>2</sup> poi. ....	..	..	30	36	..	..	3	28	57	7	
4666 F <sub>2</sub>	4482 <sup>2</sup> poi. ....	1	..	37	70	..	..	..	..	35	1	
4667 F <sub>2</sub>	4482 <sup>2</sup> + .....	81	40	..	13	..	..	..	..	..	13	
4668 F <sub>2</sub>	4482 <sup>2</sup> + x poi. ....	29	34	11	25	..	..	11	14	..	9	5 ♀ 3 ♂ poi sing = px
4683 F <sub>2</sub>	4491 <sup>2</sup> poi sing. ....	..	..	20	47	..	..	..	..	54	13	♀ hairy, 4 ♂ poi sing spread
4602 F <sub>3</sub>	4664 <sup>2</sup> poi sing. ....	..	..	40	75	..	..	..	..	60	37	25 ♀ 28 ♂ poi sing all px stock
4603 F <sub>3</sub>	4665 <sup>2</sup> poi x px poi. ....	..	..	33	45	..	..	..	..	72	84	39 ♀ 72 ♂ poi sing all px; part si
4604 F <sub>3</sub>	4665 <sup>2</sup> poi. ....	..	..	52	52	..	..	..	..	20	30	All poi sing px; male not sing
4605 F <sub>3</sub>	4665 <sup>2</sup> px poi sing. ....	..	..	..	..	..	..	..	..	38	43	All px 9 ♀ 19 ♂ not sing
4606 F <sub>3</sub>	4666 <sup>2</sup> poi. ....	..	..	40	66	..	..	..	..	26	14	
4608 F <sub>3</sub>	4666 <sup>2</sup> poi sing x poi. ....	..	..	10	35	..	..	..	..	30	7	
4611 F <sub>3</sub>	4667 <sup>2</sup> + x poi sing. ....	60	54	..	..	..	..	18	22	..	..	
4620 F <sub>3</sub>	4668 <sup>2</sup> poi sing. ....	..	..	40	64	..	..	..	..	46	3	
4618 F <sub>3</sub>	4663 <sup>2</sup> poi px sing. ....	..	..	..	..	..	..	All	All	..	..	All singed stock
4621 F <sub>3</sub>	4663 <sup>2</sup> poi sing x poi sing px	..	..	..	Part	..	..	..	..	All	Most	
4660 F <sub>3</sub>	4683 <sup>2</sup> poi sing. ....	..	..	..	Part	..	..	..	..	All	Most	
4480 F <sub>1</sub>	♀ ♂ px stock. ....	..	..	..	..	25	1	54	75	..	..	px is poi
4679 F <sub>2</sub>	4480 <sup>2</sup> px poi bl. ....	..	..	..	..	35	13	28	24	..	..	px is poi
4048 F <sub>3</sub>	4679 <sup>2</sup> px poi. ....	..	..	..	..	Most	Few	Few	Most	..	..	♀ bb achi
4949 F <sub>3</sub>	4679 <sup>2</sup> px poi. ....	..	..	..	..	Most	Few	Few	Most	..	..	No achi stock
4950 F <sub>3</sub>	4679 <sup>2</sup> px poi bliat. ....	..	..	..	..	Most	Most	Few	Few	..	..	
4951 F <sub>3</sub>	4679 <sup>2</sup> px poi bliat. ....	..	..	..	..	Few	Few	Most	Most	..	..	Few ♀ achi
5066-67 F <sub>4</sub>	4948 <sup>2</sup> achi ♀. ....	..	..	..	..	Most	Most	Few	Few	..	..	Most ♀ bb achi
5160 F <sub>5</sub>	5067 <sup>2</sup> achi. ....	..	..	..	..	Most	Most	Few	Few	..	..	Most ♀ bb achi stock



One more  $F_3$  cross ought to be mentioned (no. 4619), another cross in which a heterozygous bran was mistaken for a homozygous one, both being heterozygous for px. The result was:

No. 4619 $F_3$ f. om bran	bran (')
16 ♀ 20 ♂	bran
20 ♀ 11 ♂	bran px
44 ♀ 13 ♂	-
12 ♂	poi (soft,
1 ♀	px

The parents were clearly bran px, -, poi bl - × bran px, bran -, and the expectation was: ♀  $\frac{1}{2}$  -,  $\frac{1}{4}$  bran px,  $\frac{1}{4}$  bran; ♂  $\frac{2}{8}$  -,  $\frac{2}{8}$  poi,  $\frac{1}{8}$  bran,  $\frac{1}{8}$  bran px,  $\frac{1}{8}$  poi bl,  $\frac{1}{8}$  poi bl px. The females agree with the expectation. The sex ratio is 81 : 56 = 1.4, i.e., about  $\frac{1}{4}$  of the males are missing. Clearly, poi bl and poi bl px are missing, and the - and poi classes are deficient, while too many bran males appear. A male lethal condition is present for the poi X together with homozygous second chromosomes carrying bran. The one px ♀ (fertilized when found) obviously was one of the rare crossovers bran-px fertilized by a bran px sperm.

Thus, mutation to poi bl in some of the  $F_1$  female gametes explains part of the results. Furthermore, the original male must have been a compound of bran' with no visible expression and another bran allele, bran' which in homozygous condition has the phenotype bran, variable towards plus, and in compound with bran' is more or less intermediate; with poi bl it gives the poi blist type. The linkage of bran' with px, a combination which is rather unviable, made a further analysis impossible.

Tables 138 and 139 contain data on some of the broods of generations that followed those reported in table 137, in which further alleles became visible. Table 138 shows the extraction of px bl containing the allele bs<sup>pp</sup> breeding true for an extreme px bl in both sexes but varying toward nonblistering, mostly in males. Also, there was a tendency to extra hair and bristles, which, however, later disappeared from the stock. The table shows that this condition could be found and selected in the changed stock (4490, 4681, 4958), but that the latter also contained a poi and a bran allele (at least) which produced pointed blistered and rather slender wings. These segregated in  $F_2$  (4681) in what looks like a 1 : 2 ratio for females. Actually, this turned out again to be the combination bran' px, bran' px, poi si, which we had already found to be derived from low px in this stock. The extreme px bl type also had segregated from normals in the stock (4481, table 137) and again bred true (4662, 4912). (In 4662 one pair of low px appeared which did not reproduce; it could therefore not be ascertained whether a reversal of bs<sup>pp</sup> had occurred.) Thus the bs allele bs<sup>pp</sup> had remained from the original stock with or without the new pointed and bran alleles and in the presence of px. We know already that svr<sup>po1</sup> does not show clearly in the presence of px, but that poi bl does, and so does the new allele poi si, which then could not be present in the homo- or hemizygous condition before it became visible.

Partly the same, partly different happenings occurred in the related lines reported in table 139. From a normal pair (-, 4482) from the same stock (the px bl 369 stock with return mutation, etc.) half normals, half pointed were produced with a high sex ratio in the + and px classes; px segregated normally in the + females, and in too great numbers in the poi females; there were no px males in the

poi class. About one-fourth of the poi flies in both sexes were "singel," i.e., had a singed-looking spot near the anterior edge or the tip of the wing. The females with both px and poi were blistered. Obviously, the already described combination *bran<sup>+</sup> bran*, poi si with or without px had segregated. This means that one of the - parents had been heterozygous for *bran* and px in one second chromosome, and that the other parent had been *bran bran* px (bran has no effect without poi si). Also, the mother must have been heterozygous and the father hemizygous for poi si. A similar segregation occurred in another F<sub>1</sub> from normal parents (4491). The table shows that the extracted pointed types with or without singed could be heterozygous for px, which segregated with the varying ratios already known. The combination px poi sing could be easily extracted as a true breeding stock, though a varying number of flies were not singed. (Actually, this is the origin of the *bran<sup>+</sup> poi si* combination described above, p. 356.) Pointed singed without px seemed to segregate into poi and pointed singed. But it turned out that both groups have the same genetic basis, the singed character being variable; the majority of females were usually singed, as were the minority of males, with a considerable variation in individual broods. The stock with px poi singed later changed, so far as the singed spot became a regular blister. Another line of the same was extracted (see 4489) which from the beginning had blisters instead of singed spots. Both were genetically alike, as described. Also, px poi blist was extracted and turned out, as described, to be the same combination plus plexus and *bs<sup>99</sup>*.

One more characteristic poi allele was obtained from the same stock. A pair of px flies from the same 369 stock produced (4489, lower part of table 139) a majority of offspring of the type px poi, and a minority, almost all females, of the same type and blistered. The parents must have been homozygous for a poi allele without showing it, and it might be assumed that we had again the *bran<sup>+</sup> px. poi si* combination, which would mean that the parents were heterozygous for *bran<sup>+</sup>*. (The sexual difference could have been purely phenotypical.) The px poi blist flies reproduced their type in the next generation, though part of the flies were not blistered. Four F<sub>2</sub> were bred from blistered and not blistered flies. Two of them behaved as if they were the *bran<sup>+</sup> poi si* combination (see table 139). In one, most of the females had short bristles (phenotype *bb*) and poorly chitinized (etched) abdomen, as is found with higher *bb* alleles; both traits, however, also found with deficiencies in the a px region, a type called here "achitinous" (abbr.: *achi*). These flies were very pale, with little or disheveled hair and bristles on the abdominal tergites. In another brood only a few females had this character. Selection was made for the pale flies with and without *achi*. In some of the established stocks the *achi* character soon disappeared, showing that it was not simply due to a *bb* allele. But the pale etc. phenotype turned out to be a new allele, poi dish (see p. 390), which continued breeding true with px blist simultaneously visible. Tests showed the presence of a low *bb* allele with very little compound effect with *bb*. The *achi* type therefore had required something else, probably in the *bran* region, as discussed above. Also, the presence of *bran<sup>+</sup>* was tested and proved.

The happenings in this pedigree are rather difficult to visualize. The original parents must have possessed poi si and *bran<sup>+</sup>* heterozygous, arisen in the reverted px bl stock. The allele poi dish was most probably already present as a compound with poi si, but had been overlooked in the F<sub>1</sub> males, and segregated out in F<sub>2</sub>. The

simultaneous appearance of the *bb* allele (or its becoming homozygous) may or may not have been a chance accident. Another parallel case (see p. 387) points to some relation between the two events, and the same is true for the appearance of the *achi*-enhancing condition, suspected to be located in the *arc* region, and analyzed in other similar cases (see p. 393).

Altogether, then, in the selected *px bl* stock containing *bs*<sup>20</sup> the return mutations from *px* and *bs* to + arose, and simultaneously the mutants *bran*, *bran*<sup>3</sup>, *bran*<sup>4</sup>, *poi*, *poi si*, *poi bl*, *poi dish* spread in the stock. Also, *bb* and something in the *a* region (the *achi*-enhancer) were involved. But the *bran* alleles originated in the second chromosome with *px* as well as in those with *-px*, the small distance making an ex-

TABLE 140  
STERILITY OF THE *bran-px* COMBINATION

Gen.	No.	Cross	low <i>px</i>		soft <i>bl px</i>		<i>bran</i>		Remarks
			♀	♂	♀	♂	♀	♂	
F <sub>1</sub>	581	low <i>px</i> × low <i>px</i> . .	About 50	About 50	..	2	..	..	
F <sub>2</sub>	719	581 <sup>3</sup> <i>px</i> × soft <i>bl px</i> .	55	60	11	11	..	..	
F <sub>3</sub>	962	719 <sup>2</sup> soft <i>bl px</i> . . .	.	..	6	7	..	..	
F <sub>3</sub>	1015	719 <sup>2</sup> <i>px</i> . . . . .	129	7	.	1	..	..	♂ <i>px</i> -like hetero- <i>px</i>
F <sub>4</sub>	1098	962 <sup>2</sup> soft <i>bl px</i> mass	..	..	12	5	..	..	No more offspring
F <sub>4</sub>	1158	1015 <sup>2</sup> <i>px</i> × hetero- <i>px</i>	..	..	5	7	42	39	Two others sterile
F <sub>4</sub>	1159	1015 <sup>2</sup> <i>px</i> × hetero- <i>px</i>	..	..	12	10 <sup>a</sup>	49	56	

<sup>a</sup> 3 abn. abd.

change by crossover improbable. As in former cases, it is hardly possible to relate the events to each other in a simple diagrammatical way, though there can be no doubt that such an interrelation exists.

#### d. *bran* AND *plexus* AND IRREGULARITIES AT THE TIME OF MUTATION

There is another group of facts which must be considered relevant, though no detailed interpretation could be derived from it. We have discussed above the type soft blistered, which is genetically a combination of some *bran* and *poi* alleles and which was found whenever one of them appeared by mutation in the presence of the other or whenever both mutated simultaneously. Soft blistered has always been bred without difficulty, but when it originated while *px* was still present it was practically sterile, as already mentioned. Table 140 shows such a case. The F<sub>1</sub> parents were derived from one of the stock bottles of *px bl* I in which low *px*, *px/+*, and soft *blst* flies had appeared. The offspring of the first cross were all *px* as expected, and there were two soft *blst px* males, i.e., *bran px/bran px*. No *bran* was registered among the *px* class. But this might have been overlooked if it was present in only a few individuals. The *px* parents might have been heterozygous for *bran* and homozygous for *px* and the mother in addition heterozygous for *poi*, both just arisen in the stock. The expectation had been ¼ ♀♀ *bran px*, ⅙ ♂♂ *bran px* and ⅙ ♂♂ soft *blst px*, of which only 2 ♂♂ and maybe a few overlooked *bran px* ♀♂ survived. These soft blistered males were crossed to their sisters and F<sub>2</sub> segregated into *px* and soft *blst px*. This requires that the mother be heterozygous for both *bran*

and poi. The expectation is  $\frac{1}{4}$  ♀ and ♂ soft px blist,  $\frac{1}{4}$  ♀ ♂ bran px,  $\frac{1}{4}$  ♀ ♂ px poi,  $\frac{1}{4}$  ♀ ♂ px. Pointed (i.e., *svr<sup>poi</sup>*) with px is not easily seen. No bran px were recorded, and otherwise the ratios were not perfect. Extracted soft blist px bred true, as expected, but were highly infertile. *F*<sub>4</sub> was bred from all 13 *F*<sub>3</sub> flies and only 17 *F*<sub>4</sub> flies were obtained, which latter did not reproduce at all. Thus bran with poi, just arisen in the presence of px, is almost sterile. The px flies of *F*<sub>2</sub> gave in *F*<sub>3</sub> only px daughters and px soft blist sons with a sex ratio of 15 : 1. This indicated male lethal recombinations of the first, and probably of the second and third autosomes, as was observed before when these mutants appeared. Simultaneously, the few males had lost px in one chromosome; their phenotype was that of px/+, and so was part of their offspring. This in *F*<sub>4</sub> consisted of about  $\frac{5}{6}$  bran and hetero-px ♀ and ♂, and  $\frac{1}{6}$

TABLE 141  
SELECTION FROM CHANGED STOCK px bl I

Gen.	No.	Cross	+		low px		soft bl		bran		Remarks
			♀	♂	♀	♂	♀	♂	♀	♂	
<i>F</i> <sub>1</sub>	579	+ × soft blist (stock).	57	54	..	..	..	.	..	..	Some ♀ ♂ + hetero-px type
<i>F</i> <sub>1</sub>	580	+ × soft blist (stock).	35	31	7	13	..	..	..	..	Some ♀ ♂ + hetero-px type
<i>F</i> <sub>3</sub>	715	579 <sup>2</sup> ♀ hetero-px, ♂ +.	43	12	..	..	..	25	66	36	+ = hetero-px
<i>F</i> <sub>2</sub>	716	579 <sup>2</sup> ♀ hetero-px, ♂ +.	50	43	16	15	1	15	.	..	3 ♀ 3 ♂ px bl
<i>F</i> <sub>2</sub>	717	579 <sup>2</sup> + .....	..	..	..	..	2	35	61	40	
<i>F</i> <sub>3</sub>	973	717 <sup>2</sup> bran .....	..	..	..	..	..	..	All	All	
<i>F</i> <sub>3</sub>	974	715 × 717 bran .....	..	..	..	..	..	..	All	All	Stock

soft blist, with a normal sex ratio. Two of the *F*<sub>3</sub> px/+ males were sterile. In both cases the two *F*<sub>3</sub> (1015) parents bred true to bran, which they had not shown, and they were heterozygous for pointed, which, however, appeared in *F*<sub>3</sub> only in  $\frac{1}{6}$  instead of  $\frac{1}{2}$  of the offspring. But bran had become clearly viable by becoming heterozygous for px! Bran was not further selected, since it had been isolated simultaneously from other crosses. This is to be regretted because thus we do not know which allele was present. A detailed interpretation is certainly difficult, but we see again relations between the happenings at the px, bran, and poi loci involving mutation and what looks like return mutation, with the simultaneous appearance of lethal male classes.

In the same px bl stock from which the low px of table 140 originated there appeared wild-type flies and soft blistered males. The latter turned out to be the bran-poi combination without plexus and completely viable. Table 141 shows that wild type and soft blistered could be heterozygous for px and that wild type did not contain pointed. Soft blistered is of course the bran-poi combination, but again a few additional complications were present in connection with the appearance of bran and poi and the loss of px and bs from the px bl stock. Table 141 shows the first generations.

In *F*<sub>1</sub> px segregated once, in which therefore soft blist had been heterozygous. *F*<sub>2</sub> 579 showed that here also one of the parents had been heterozygous. We see that two of the *F*<sub>2</sub> segregated bran without px, which had been introduced by the soft

TABLE 142

ORIGIN OF BRAN WITH AND WITHOUT px

Gen.	No.	Cross	+ (soft)		px		soft bl		soft bl px		Remarks
			♀	♂	♀	♂	♀	♂	♀	♂	
F <sub>1</sub>	578	px × soft blist.....	15	13	..	..	7	14	..	..	+ = hetero-px
F <sub>2</sub>	712	578 <sup>3</sup> soft blist.....	7	5	3	3	26	29	6	6	1 ♀ soft blist is sooly
F <sub>3</sub>	713	578 <sup>3</sup> soft blist.....	..	3	3	1	36	40	19	17	1 ♀ 1 wing slender
F <sub>3</sub>	714	578 <sup>3</sup> soft blist.....	71	..	21	1	3	24	1	3	1 ♀ high px
											1 ♀ px blist
											2-1 ♀ scute
F <sub>3</sub>	999	713 × 715, soft bl.....	..	..	..	..	All	All	2	6	4 more F <sub>3</sub> from soft bl px sterile
F <sub>3</sub>	1009	712 <sup>3</sup> soft bl (♀ dp type) ..	..	..	..	..	..	..	3	3	Normal fertility
F <sub>3</sub>	1011	712 <sup>3</sup> soft bl.....	..	..	..	..	..	..	..	..	Only few
F <sub>4</sub>	1150	999 <sup>3</sup> soft bl.....	..	..	..	..	All	All	..	..	Only few
F <sub>4</sub>	1154	1009 <sup>3</sup> soft bl.....	..	..	..	..	..	..	All	All	Normal fertility
F <sub>4</sub>	1156	1011 <sup>3</sup> soft bl.....	..	..	..	..	..	..	..	..	Normal fertility
F <sub>6</sub>	1206	999 <sup>3</sup> soft bl.....	..	..	..	..	All	All	..	..	Normal fertility
F <sub>6</sub>	1318-19	1206 <sup>3</sup> dp or not .....	..	..	..	..	All	All	..	..	Stock
F <sub>6</sub>	1250	1150 <sup>3</sup> .....	..	..	..	..	All	All	..	..	

blister father in one of his second chromosomes not containing px. Thus both bran and soft blister without px were obtained and produced perfectly fertile stocks, as opposed to the same combinations with px. But the  $F_2$  ratios again were strange. (The extracted bran and soft blister stocks behaved in accordance with expectation in outcrosses, though the ratios for the bran and soft blister classes were not always perfect.) No. 715 segregated as if the cross had been  $\text{bran}/+ \times \text{poi}/+ \times \text{bran}/\text{bran}, +/+$ . The expectation is:  $\varnothing \frac{1}{2} \text{ bran } \frac{1}{2} +$ ;  $\sigma \frac{1}{4} \text{ poi } \frac{1}{4} \text{ bran } \frac{1}{4} \text{ soft blister } \frac{1}{4} +$ . If we are willing to accept the numbers in general, the result is nearly obtained, but all males poi and not bran are missing, i.e., lethality in the presence of heterozygous bran px, and that only in the simultaneous presence of poi. Though one of the parents must have been bran, it was not recorded, though so unobtrusive a feature as the heterozygous plexation had been noted. It is therefore hard to believe that bran had been overlooked.  $F_2$  717 must have had both parents bran, and finally, 716 segregated soft blister and also px and no bran. As it is inconceivable that bran was not seen in 716 but was seen in 715 and 717, checked within the same hour, we must assume that here as well as in a former similar case bran was somehow suppressed in the phenotype (or had arisen as a mutation in the germ line only?). No final explanation can be offered. The table shows in 716, 717 a few females soft blistered which are clearly not expected (all females heterozygous for poi). There is a strong suspicion that once more the bran locus had mutated.

One more pedigree may be added in which both soft blister and the same with px appeared in the same brood, the former being viable and selected for establishment of a stock which has bred normally for six years since, whereas the latter was almost sterile. From the same bottle from which the parents of the former crosses were obtained, a rather low px female was crossed to a soft blistered male. Table 142 contains the results.

$F_1$  578 shows that the px mother must have been heterozygous for bran (second chromosome) and poi (first chromosome) in order to produce  $\frac{1}{2}$  soft bl offspring, i.e., the cross was  $\text{px bran}/\text{px}, \text{poi}/+ \times \text{bran}/\text{bran}, \text{poi}$ . Four classes of offspring were expected: (1)  $\text{px bran}/\text{bran}, \text{poi}/\text{poi}$  ( $\sigma$  poi); (2)  $\text{px bran}/\text{bran}, \text{poi}/+$  ( $\sigma$  +); (3)  $\text{px}/\text{bran}, \text{poi}/\text{poi}$  ( $\sigma$  poi); (4)  $\text{px}/\text{bran}, \text{poi}/+$  ( $\sigma$  +). Of these, no. 1 (soft bl) and no. 4 (+) alone were present, i.e., the combination  $\text{px bran}/\text{bran}$  (= broad) was not viable without poi chromosomes, and the combination  $\text{px}/\text{bran}$  was not viable with poi chromosomes.  $F_2$  from both parents no. 1 (soft bl) ought to be all soft bl with one-fourth px. Actually (712), in both classes, without and with px, about one-fourth (a little less) of the flies were neither soft bl nor broad, but + and px. This would require that another chromosome be heterozygous in the parents for something which suppresses the bran effect (and therewith soft bl). A similar feature has already been described in the former case. Unfortunately, no analysis of these extra classes was tried at the time, but if bran is a small deficiency produced by nonreciprocal translocation, the duplication would act as an inhibitor and would be expected to be present when bran is newly formed. Another  $F_2$  (713) ought to have given the same results. Actually, there was a segregation of not  $\text{px} : \text{px} = 2 : 1$ , and the two unexpected classes + and px contained only a few individuals. The ratio of  $112 : 7 =$  very near  $15 : 1$  may be significant, but it is possible also that the seven not soft bl flies were the product of an autosomal crossing over, involving the inhibitor, though this is difficult to imagine. The third  $F_2$ , no. 714, was stranger

still. There were almost no soft bl females, which looks as if all females but  $\pm$  out of 95 contained the inhibitor for bran. The males were all but one soft bl, but  $\frac{3}{4}$  of the males were missing. This shows that the X chromosome was also involved. It is very difficult to give an interpretation of these data in concrete terms, as the unexpected classes were not analyzed at this stage. One thing is certain, namely, that a complicated relation between the second chromosome, involving px and bran, the first chromosome, involving poi, and some other locus and at least another autosome were involved in the happenings which produced low px and soft bl (= bran - poi) out of px bl. It is further remarkable that no broad angular = bran was segregated. In both former groups bran was obtained in  $F_2$  or  $F_3$  after crossing soft blistered to one of the other types from the same line. As the original mother here had been px, as was also the case in the first group, the px chromosome alone cannot be responsible. Soft blistered with px produced from these crosses was almost sterile (1009, 1011, 1154, 1156). But one soft blistered female (713) was crossed to a soft blistered male of unrelated origin (715, a cross which had produced bran; see table 141), and from this cross a fertile stock could be immediately established in the absence of px. Again, the px chromosome was involved in the sterility and unviability of soft bl containing this chromosome as well as the suppressor of broad, the latter obviously being involved both in the sterility and in the unexplainable ratios. Though we cannot propose any concrete interpretation, I can hardly see that one could be derived which would not be based upon the consequences of small translocations involving probably three chromosomes, translocations of the type actually found in the genetic analysis presented above.

When the pointed singed flies had appeared, as reported in the last section, after outcrossing, the combination bran px/bran px was segregated (see p. 494). This was almost sterile and a stock soon died out. As the X chromosome (derived from Oregon wild) did not contain poi, it follows that the sterility just discussed for the soft blist px combination is due only to the bran px combination, and we point again to the salivary analysis of these loci.

Only one more case of mutation within a stock involving the known loci may be described. In a true-breeding soft blistered stock (= bran/bran; poi), no. 5085, a single female was found with one wing normal, the other truncated, and, in addition, a dark trident on the thorax. This female was crossed to a pointed male and all offspring were pointed, but half of the sons were missing. In  $F_2$ , from this, not only poi and soft blistered segregated as expected, but also bran, which turned out to be a new bran allele which, with poi, produces long but soft and blistered wings. Bran could only segregate if some poi gametes had mutated back to normal. The dark trident also segregated and turned out to be an ebony allele. Thus a new bran allele had appeared in one female, together with ebony and, as it seems, a mutation from poi/poi to poi/+. The sons with the return mutation X (+) were lethal. But in the offspring of the daughter, heterozygous for the poi from the stock and a standard poi, a part of the poi X mutated back to normal, but apparently only in the gametes simultaneously containing bran (bran/bran, poi/+ flies and not bran/+ or +/+, + flies).

#### e. MUTATION IN THE LINES $svr^{poi}$ AND px bl

We have reported above that in an inbred stock of  $svr^{poi}$  a bran or a soft blist (bran/bran, poi) individual is occasionally found. Once we succeeded by chance

in finding a  $svr^{poi}$  female which had become heterozygous for bran by mutation, with simultaneous mutation of  $svr^{poi}$  into  $svr^{poi+}$ . Many crosses of  $svr^{poi} \times bw\ sp\ ba$  had been made with normal results. But in one such cross half of the sons had soft and partly singed wings. We already know (see table 74 of phenotypes) that the allele  $svr^{poi+}$  in the presence of heterozygous bran produces such a wing in some individuals, the homozygous combination being soft blistered. Actually,  $F_2$  segregated, according to expectation, bran and soft blistered and soft singed like  $F_1$ . (It is remarkable that the segregants combining  $bw\ sp\ ba$  with  $poi\ s$  were extremely blistered, blisters never being found in this  $bw\ sp\ ba$  stock). Thus, the  $poi$  mother had been heterozygous for the mutants bran and  $poi\ s$ , both of which had not been found among the other  $poi$  flies.

We have mentioned above that the mutant  $svr^{poi+}$  has occasionally cropped up in the  $px\ bl$  stock and spread through it. One should expect the same for bran. But whereas bran homozygous together with  $px$  is poorly viable, it might not survive at all in a stock bottle. Special tests made for the presence of bran in  $px\ bl$  were

TABLE 143

	Cy Sb	Cy	Sb px bl (few not bl)	px bl (few not bl)	Sb	Cy px
♀ . . . . .	25	10	12	8	1	..
♂ . . . . .	24	24	16	8	3	1
Exp. . . . .	4	2	2	1	C.o.	C.o.

always negative. Only once, namely, at the time when  $poi\ s$  had spread in the stock, as described above, was bran discovered by chance. A considerable number of crosses  $px\ bl \times Cy/Pm, Sb/H$  were made in both directions and with blistered as well as not blistered flies. From these, many different  $F_2$  were derived. In one of them,  $F_1$  569 Cy, Sb  $\times$   $F_1$  562 Cy, Sb, both from highly blistered parents (mother in 569, father in 567), most of the segregating offspring with the original second chromosome containing  $px$  were again plexus and blistered, which we know is not the case in other  $F_2$  involving  $px\ bl$ . The actual segregation is given in table 143. The considerable amount of crossing over between Cy and  $px$  is unusual. The plexation was extreme, since Sb usually acts as an enhancer. The wings were long, not short as in soft blistered. In the next generation from  $px\ bl$  flies (with and without Sb) only plexus blistered flies were obtained, but now they had low plexation with or without Stubble. These bred true for soft blistered plexus, which was more or less sterile and a stock died out. This shows that both original parents had not only contained  $poi$  but were also heterozygous for bran, which explains their stronger blistering. By chance, one of the  $F_2$  bred from both  $F_1$  was made from  $F_1$  parents Cy/bran  $px$ , as the further offspring revealed. We have found by tests that the enhancing action of the Sb chromosome upon  $px$  expression is due to a simultaneously present inversion. Obviously, this enhancing action also prevented the shortening of the  $px$  bran  $poi$  wings. It cannot be explained why the enhancing action was lost in  $F_3$  and  $F_4$  and the soft blist  $px$  type became visible. There is a possibility that another  $poi$  or bran allele was involved. We remember that bran  $px + poi$  is phenotypically soft blistered plexus, and bran  $px + poi\ s$  is soft blistered spread plexus. The some-



TABLE 144  
CROSSES WITH yellow INVERSION AT THE TIME OF RETURN MUTATION OF px bls

Gen.	No.	Cross	♀	♂	New mutants
F <sub>1</sub>	3696-98	poi × y-Inv.	As expected.	As expected.	
	3710-11	poi h × y-Inv.	As expected.	As expected.	
F <sub>2</sub>	3746-47	px <sup>a</sup> × y-Inv.	29 px (15 bl), 46 +	33 px, 22 +	brun <sup>dp</sup>
RF <sub>2</sub>	3812	3696 <sup>2</sup>	78 as expected, 1 poi:dp.	87 as expected.	
RF <sub>2</sub>	3940	3740 + × px <sup>a</sup>	1/2 px, 1/2 + <sup>b</sup>	1/2 px, 1/2 + <sup>b</sup>	
RF <sub>2</sub>	3941	px <sup>a</sup> × 3746 +	45 extr. px bl, 64 "low px" <sup>1</sup>	49 extr. px, spread, part blist., 28 poi bl, 23 +	poi bl, brun
RF <sub>2</sub>	3942	3747 px bl × px <sup>a</sup>	103 extr px (1/2 blist.)	50 extr. px, 18 ditto y, 16 y low px.	2 ♀ mut. px/px →
RF <sub>2</sub>	3943	px <sup>a</sup> × 3747 px bl.	47 ♀ px (part bl), 2 ♀ low px.	55 px.	px/+ blist
RF <sub>2</sub>	4528-29	Y × 3911 px spr bl.	Short bristles.	19 spr bl, 21 + blist, 6 +	
RF <sub>2</sub>	4530-31	Y × 3941 +	Normal.	Normal.	
RF <sub>4</sub>	4693	4529 <sup>2</sup> ♂ spr bl.	Normal.	1/2 +, 1/2 blist.	
RF <sub>4</sub>	4695	4530 <sup>2</sup> ♂ +		+ 1 px.	px return
RF <sub>4</sub>	4696	4530 <sup>2</sup> ♂ +		47 +, 4 px.	px return

## SELECTION IN I(1) ypx bl

F <sub>1</sub>	3949	y + from bottle.	67 +, 1 px bl, 3 low px, 3 dwarf, 1 dwarf low px.	60 +, 5 low px, 5 dwarf, 1 dwarf low px.	dwarf and px seg.
F <sub>2</sub>	4532	3949 <sup>2</sup> low px.	61 + and short bristles.	77 + and bristles short.	
F <sub>2</sub>	4533	3949 <sup>2</sup> dwarf px.	6 px and px bl, norm. br.	18 px bristles norm.	
F <sub>2</sub>	4534	+	62 high px (13 blist). 49 +	48 high px; 30 +	
F <sub>2</sub>	4535	+ very large.	+	+	
F <sub>3</sub>	4707 etc.	4532 <sup>2</sup> +	Not px : px 3 : 1.	Like ♀	
F <sub>3</sub>	4728 etc.	4532 <sup>2</sup> px.	All px and blist.	Like ♀	

<sup>1</sup> y stock; px extreme from poi × poi h  
<sup>2</sup> px/+ like low px.

what different phenotype here may therefore have been based upon other alleles which could not be tested on account of the sterility of the combination. The pointed allele appeared more like soft in such combinations (without bran, px, Cy) as could show it.

We have reported above the origin of the  $\text{Inv}(1)y^{px\ bl}$  as a single male in the px bl stock, and we stated that in the homozygous stock of this inversion individuals appeared heterozygous for px and bs (by return mutation) from which an  $\text{Inv}(1)y^{px\ bl}$  stock without px and bs was isolated. When this happened a not px pair but  $\text{Inv}(1)y^{px\ bl}$  from the bottle in which the return mutation had occurred was bred. The offspring consisted of 127 +, 9 low px, 8 dwarf, 2 dwarf low px (both sexes alike). The parents were obviously heterozygous for px, but the segregation for px was about 12 : 1 instead of 3 : 1, and, in addition, dwarfs appeared. This shows that the return mutation was hardly the simple event implied by the name. A pair of the low flies (all y-Inv) produced 61 ♀ 77 ♂ normal, but with bristles varying from shortened to very short; further, 6 ♀ px (3 blist) and normal bristles, 18 ♂ px, also normal bristles. What had looked like homozygous px was again heterozygous, and  $\frac{1}{11}$  ♀ and about  $\frac{1}{5}$  ♂ px segregated, the not px flies having abnormal bristles—again, not a clear or normal behavior. The px dwarfs also produced a segregating offspring, all large, not giant, all with normal bristles, namely, 62 ♀ px (12 blist) 48 ♂ px, 42 ♀ 29 ♂ +. Crosses of + with dwarf were normal. In the following generations px segregated, where heterozygous, quite normally, and + bred true or was px/+. Extracted px had the same phenotype as was originally found in the stock.

Simultaneously, a y-Inv male was crossed to an extreme px from the stock which had been derived by mutation from  $\text{poi} \times \text{poi h}$  (see p. 480) and which did not contain any more poi, but  $\text{bs}^{pp}$ .  $F_1$  segregated into px with and without blisters and normal or hetero-px flies, one-half of the males of the latter class missing. The father thus had been px/+. There were no poi ♂ or ♀. Normal females were backcrossed in both directions to the px parental line. The offspring segregated into strong px and heterozygous px of a much higher grade than normal, a result of the simultaneous presence of the  $\text{bs}^{pp}$  derived from the mother. Moreover, about one-fourth of the males were typical pointed blistered not px flies, which requires the presence of bran and poi bl, neither of which had been present in either parent. One-half of the males were strong px and spread, some blistered. (In a reciprocal cross, also, two females among 49 showed a return mutation to px/+. ) A test of these males with y females produced spread or not spread and blistered males, whereas all females had short bristles. The X chromosome thus did not contain poi, but another sex-linked mutant causing the spread blistered wings. This type was inherited as a simple sex-linked character, but was later lost. The normal brothers of the spread blistered males from the first  $y \times$  spread blistered cross gave only normal sons with y mothers. But twice a few px males segregated, once 4 among 51 (2 of them combined with the marker ey) and once 1 among the same number; thus px had again returned. Finally, a cross of  $\text{poi} \times$  the same y Inv, which had become heterozygous for px, threw in  $F_2$  1 poi : dp ♀ among 79, meaning a mutation to bran<sup>pp</sup>. Further tests of all these features did not furnish any more information. Table 144 presents the foregoing data.

These detailed descriptions may suffice. Corresponding cases contained only in the tables followed the same pattern.

TABLE 145  
Origin of rudimentary

Gen.	No.	Parents	+		pol		rud		low px		px bl	
			♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
P	4203 B	px bl.....	..	..	..	..	..	1	..	..	All	All
F <sub>1</sub>	4474 B	px bl x px bl.....	136	70	..	..	..	37	18	19	..	..
F <sub>2</sub>	4610 B	+ x +.....	185	80	..	..	..	55	..	..	..	..
F <sub>2</sub>	4612 B	+ x +.....	151	..	..	65	..	1	..	..	..	..
F <sub>3</sub>	4613 B	+ x +.....	8	3	..	..	..	..	..	..	..	..
F <sub>3</sub>	4764 B	4612 <sup>♀</sup> x + x ♂ r.....	77	..	..	43	29	34	25	15	..	..
F <sub>3</sub>	4765 B	4612 <sup>♀</sup> + x +.....	199	67	..	?	..	49	2	..	..	..
F <sub>4</sub>	4988 B	4764 <sup>♀</sup> px x px.....	..	..	..	..	..	24	77	42	..	..
F <sub>4</sub>	4977 B	4764 <sup>♀</sup> + x poi.....	73	35	..	19	..	41	43	29	..	..
F <sub>4</sub>	4978 B	4765 <sup>♀</sup> + x r.....	28	53	..	13	..	71	70	..	..	..
F <sub>5</sub>	5178 B	4988 <sup>♀</sup> px poi x px spr bl..	..	..	..	..	..	10	37	12	..	..
F <sub>5</sub>	5173 B	4977 <sup>♀</sup> px Mass.....	..	..	..	..	..	14	132	33	..	..
F <sub>6</sub>	5348 B	5178 <sup>♀</sup> px x px.....	..	..	..	..	..	16	110	125	..	..
F <sub>6</sub>	5352 B	5178 <sup>♀</sup> px spr bl x r.....	..	..	..	..	13	14	10	6	..	..

poi partly + ?  
1 ♂ dwarf

+ and poi not classifiable  
9 ♀ 7 ♂ px poi, 1 ♀ 3 ♂ px spr bl

57 ♀ 27 ♂ px spr bl, 5 ♀ broad round  
4 ♀ 3 ♂ poi px, 9 ♀ px spr bl  
85 ♀ 69 ♂ px high, 10 ♀ 15 ♂ px spr bl  
34 ♀ 41 ♂ px spr bl

## f. THE MUTANT rudimentary

A short discussion of the origin of rudimentary will show features comparable to those discussed thus far. The first appearance of rudimentary as a single male out of 90 simultaneously with the complete change of the *px bl* type has been recorded on p. 294, and also in table 1. Table 145 contains the data for the first generations, the first rudimentary male not having produced any offspring. (Most of the data have already been given in other tables.) A new appearance of rudimentary males in the offspring of the same line occurred twice later. From no. 4610 B a true-breeding normal (+) line was isolated. Four generations later, two females from this inbred line, which had not thrown any rudimentary males, were crossed to a second-chromosome marker stock (quintuple) and also to a third-chromosome one (*rucuca*). No rudimentary flies appeared in  $F_1$ . But two of the backcrosses of  $F_1$  females with the marker-stock males produced among a large number of males a few rudimentary, namely, in the second-chromosome cross 1 ♂ rudimentary and speck, in the third-chromosome cross 4 rudimentary males in the classes triple, *ru h*, and *ru c*. The crossover classes might be chance, but it is remarkable that both *sp* and the *ru h* region are also involved in the twin mutant pointed (see above). A few more instances of the reappearance of a few rudimentary males in the descendants of the *px bl* stock have been found. As an example I mention a cross  $\bar{y} \times px bl$ . In  $F_2$  (no. 300) one rudimentary male among numerous normal ones was found. In other such cases the rudimentary male did not breed and therefore could not be counted with certainty as a mutant. But it is remarkable that, unlike *bran* and *poi*, rudimentary has not reappeared frequently, though it is regarded generally as a rather unstable locus.

As table 145 shows, the first rud males appeared after the manner of a mutant simultaneously with the disappearance of *px bl* from a whole brood. But as three out of four broods from normal parents of this  $F_1$  produced rudimentary sons, many of the females in  $F_1$  must have been heterozygous for *rud*, which, then, was in some way produced in many gametes of the father of  $F_1$  but only in one egg of the mother, though it is also possible that only the mother was involved and only one *rud* son survived because of some lethal condition. Actually, one-half of the + males are missing in  $F_1$ , which may have been the *rud* males, lethal because of something else in the X chromosome, which was removed in one male by crossing over. It is remarkable that in one of the  $F_2$  the rudimentary males are only  $\frac{1}{2}$  of the normal brothers, again indicating a lethal class. In  $F_3$  from the wild type in  $F_2$ , half of the males are missing in the *rud* class as well as in the plus class. In  $F_3$  from +  $\times$  *rud*, the plus female turned out to be heterozygous for *rud*, and correspondingly *rud* females and males are produced, but not in one-half of the offspring; actually more than one-half of the expected rudimentary individuals are missing. This might seem to be a question of viability, but we shall see later that this is not the case. Some of the data of table 145, such as  $F_4$  4978, show the expected one-half rudimentary males, so that some combination with something lethal is involved, presumably a combination with another chromosome.  $F_6$  5348 is another instance. Here the rudimentary males are only about  $\frac{1}{4}$  of the males; and in  $F_4$  4977, again the *rud* males count exactly one-half of the expected number. It is hardly possible to reconstruct from these facts the first appearance of rudimentary. If we call it a

mutation in one of the X chromosomes of the mother of the line, we are confronted with the fact that other things happened simultaneously which made the same X chromosome unviable in certain combinations with one or more autosomes. One more fact ought to be mentioned. When rudimentary males were first produced in numbers (4610, 4612), each of the two broods contained one rudimentary male with some extra features never since observed, namely, a pathological combination of wing, leg, and antenna-like structures on one side of the body. (Both males were sterile.) This might have been a chance occurrence, but it might also have been a rarely surviving deficient or duplicated condition found in connection with the whole process of "mutation."

When it first appeared, rudimentary still contained the plexus blistered constitution which otherwise had disappeared. The rud individuals in table 145 ought, therefore, to be classed also under px bl. When bred with attached X yellow ( $\bar{y}$ ), px and px bl segregated among the  $\bar{y}$  flies. A first test with standard rudimentary ( $r$ ) showed the new rud to be an allele of  $r$ , which ought to be called  $r^{px\ bl}$ .

The other important fact regarding the origin of rudimentary is its production simultaneously with pointed during the same set of changes. Two of the three  $F_1$  - which threw rudimentary sons simultaneously produced one-half pointed sons. These plus females, liberated from the px bl phenotype by some change, then had one X chromosome with pointed, one with rudimentary. But we shall see that the rud chromosome also contained pointed. A poi type in the mother was possibly overlooked since the mutant had not yet been discovered. But it is also possible that both poi and rud had originated simultaneously in one chromosome for which the mother was heterozygous and therefore normal, and that both loci, distant 57 units, showed almost complete crossing over with only a few noncrossover normal flies. It is further possible that the mother of both poi and rud had been a crossover fly from a crossover in a former generation in which heterozygous poi and rud had arisen in the same chromosome. (It must be said now that a ♀ poi/poi  $r$  looks like poi, but females and males poi and rud both homozygous or hemizygous are phenotypically rud).

The mutant has since been bred over  $\bar{y}$  for many years. For a long time the type was constant; later it varied considerably. At present only one stock remains as it was, with short blistered wings, still containing pointed. Other stocks show perfectly normal wings, but outcrossed to heterozygous  $r/+$  females they produce rudimentary daughters. Obviously another allele of  $r$  has arisen with long wings and better viability. Modifiers are not responsible for this type. (Rudimentary alleles with normal wings have been described before.) In other bottles other types appeared, such as long dumpy-like wings, wings half-long and lancet-like with isolated tufts of marginal hair, and others. We shall return to these types below. Only plexus and blistered could be removed from the rudimentary wings, but blistering remained in the short-winged type owing to the presence of pointed. The phenotypes of combinations of rudimentary with pointed and bran are:

bran/bran, poi  $r^{px\ bl}$ /poi  $r^{px\ bl}$  = short rudimentary blistered

bran/bran, poi/poi  $r^{px\ bl}$  = soft blistered

poi  $r^{px\ bl}$ /poi  $r^{px\ bl}$  = rudimentary short and blistered; but some flies with an epistasis modifier vary from pointed via pointed with a few marginal hair at tip missing, via pointed with a slightly sinuated tip, and via all transitional stages of truncation to truncated or dumpylike  $r^{px\ bl}/r^{px\ bl}$  = dumpy or even normal

Rudimentary together with pointed gives the short blistered rudimentary wing. It thus shows an epistasis over pointed, as is found also with other loci with a truncating effect, such as *bran*<sup>9</sup>, and it also produces the characteristic blistering effect. The latter is even visible with low penetrance in the heterozygote *poi*, *poi r*. A semi-dominant modifier was found which shifts the epistasis toward pointed with all transitions which resemble (though they are not completely identical) the *ll-bran* combinations described above. Rudimentary has many other remarkable features, but as they are also found in other rudimentary stocks their discussion does not belong to the present subject and will be presented independently.

#### 4. MUTATION AFTER OUTCROSSING *px bl* AND ITS DERIVATIVES

Though the stocks of *px bl* and its derivatives were carefully watched, mutation in the stock bottles was rarely found, if we consider now other mutants than those at the *svr*, *arc*, *px*, and *bs* loci. This does not mean much, since recessive and poorly viable mutants are swamped in stock bottles. Dominant and rather viable mutants were also absent. There were also many long series of inbred pair matings made with different stocks, and again no mutants were found, which shows that the general mutation rate apart from the phenomena already described is the usual low one. But when *px bl* and its derivatives were outcrossed or crossed among themselves, dominant and recessive mutants appeared in significantly high numbers and in many cases in a way which excluded their presence in heterozygous condition in the stock. A quantitative evaluation is, however, extremely difficult, except for dominants, because many of the recessive mutants have incomplete penetrance and therefore may have been present before they were found. Moreover, the possibility of long-continued breeding of heterozygotes with normals is not excluded in an experiment of normal size not planned with special detecting methods.

To describe one case in detail: The only mutant not affecting the loci studied thus far which was isolated from *px bl* stock in many years of breeding is a small inversion with one break in the yellow region (see salivaries, text fig. 4),  $T(1)y^{px\ bl}$ , with the appearance and behavior of a yellow allele (see p. 398). It was found as a single male in *px bl* stock. We have already described how it reverted later from *px* to normal without a change in the X chromosome, and a stock was kept as *y-Inv* without *px*. In the hope of reproducing this event we bred for six generations 30 pairs in brother-sister matings, altogether about 45,000 flies, without a single mutant appearing. Nine months later a series was started by crossing 10 pairs of *Bld-T* with this *y-Inv* and backcrossing in each generation heterozygous females recognized by *Blond*, with *y-Inv* males. In ten generations totaling 3,263 ♀ 3,774 ♂, a mutant "spiny legs" (*sple*) was segregated four times, in irregular numbers. In  $RF_4$ , 4 ♀ 6 ♂ among 59 ♀ 58 ♂ appeared. In another  $F_7$  they appeared in Mendelian numbers. This  $F_7$  was derived from the same  $RF_2$  and a divergent line thence; a third time, in  $RF_6$ , 7 ♀ 5 ♂ appeared among 33 ♀ 34 ♂; this, again, was derived from  $F_4$ , which had given the second case in  $F_7$  in another line of origin. Finally, a fourth appearance was only 2 ♀ among 70 in  $RF_6$ , which in  $RF_4$  had been branched off the line of the last case. Now, in the first case and the stock established from it the character had very little penetrance; in the second case it was expressed very strongly; the same was true in the third case; and in the last case it was extremely strong, with additional features (see description below). It turned out that three different

alleles were involved, a very low, a medium, and a high one. Now, the common ancestor of all four was one  $RF_2$  which gave rise to three out of ten lines; but by the chances of breeding in following generations this line was selected for propagation. Now, had the original pair been heterozygous? Was the mutant carried along and only rarely given a chance for segregation? This is hardly probable. In ten generations, which in time became more closely related by selection of one of the original lines, only 4 among 83 broods (with an overall expectation of 21 in case of former presence of the mutants) segregated the type. Furthermore, the expression of the type was very strong on two occasions and could not have been overlooked otherwise, and in one of these cases, the extremest type, only 2 among

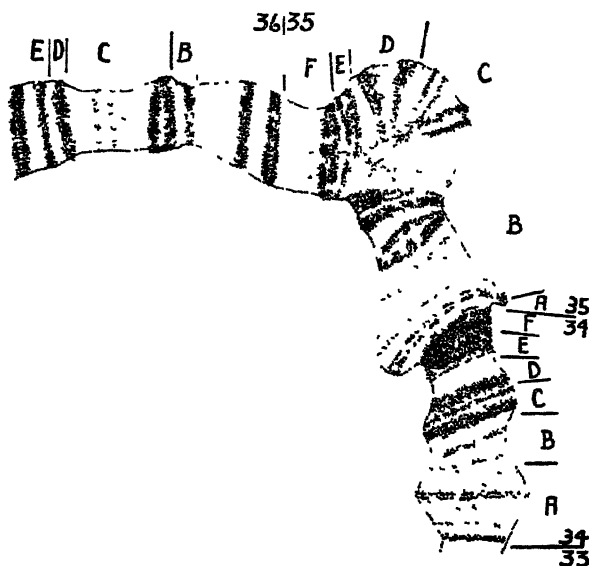


Fig. 4. Black deficiency (dominant black). M. Kodani del.

70 ♀ appeared. Thus the case is rather good for two or three independent mutations after crossing.

There are a few more remarkable features of this case. We add at this point that the localization of the mutant called *sple* gave it a location of 5.5 units to the right of black in the second chromosome, i.e., at 54. In *DIS* 16, P. T. Ives described a mutant tarsae (*sic*!) irregular-ti at 2-55.9 found in wild type. It is possible that our *sple* is allelic with this. A crossover experiment with *c* (75.5) gave 16.2 per cent, i.e., location at 59.3. (The latter test was made with 1,189 flies.) The lower alleles were not checked immediately for the salivaries. Meanwhile, the stocks of the lower alleles had become phenotypically identical with the highest allele (disheveled hair, etc.). A selection of modifiers is improbable since the original tests had agreed with multiple allelism. Thus we must assume that the high allele had originated as a mutant in the low stock and spread here. Actually, this allele shows simple segregation without much variability, thus demonstrating its nature of a multiple allele. The salivaries of this allele were, so far as could be ascertained, normal both to the left and the right of the spindle fiber (s.f. at 55).

In the same line another mutant appeared which could be proved to appear repeatedly, namely, a dominant. The phenotype was similar to dachs but differed in the extreme hairiness of the dachs legs (possibly a combination with spiny legs) and broad, blunt, arched, and spread wings. The mutant was so weak that it always died in the mating vials without offspring. It appeared in the tenth generation of the series as 18 ♀ 19 ♂ among 60 ♀ 70 ♂, both Bld or  $\gamma$ -Inv otherwise. This looks more like a recessive. But when, soon afterward, a mass backcross was made of standard yellow with our  $\gamma$ -Inv backcrossed to yellow, 13 among 603 flies were of exactly the same dachs type, thus revealing its dominance (all chromosomes were heterozygous with those from standard yellow). Some time later, 1 ♀ 3 ♂ were found in the  $\gamma$ -Inv stock. This, then, is certainly a recurrent dominant mutant. All the facts in this case point to a rarely realized or surviving deficiency duplication from translocation. No test was possible, as the type never occurred in crosses with recessive markers.

Furthermore, in the line of crosses under discussion, nicked wings were found which were inherited in an irregular way with low penetrance, and which could not be isolated. These are, then, five mutants produced in numbers which cannot be determined reliably in about 7,000 crossbred individuals, as opposed to none among 40,000 in the controls.

We had found a number of comparable cases, actually almost in each case in which *px bl* or one of its derivatives was crossed, and many generations of the offspring were bred (for other purposes than producing mutations). The following example will be given in detail because dominant mutants make it favorable for study (see table 146). This line begins with two reciprocal crosses of pointed blistered stock, long inbred, to Blond translocation (nos. 3920-23). *Poi blist*, we remember, is the combination of *bran* in the second chromosome with *svr<sup>poi bl</sup>* in the first chromosome.

In  $F_2$ , two certain and one probable mutant appeared, namely, dachs (*d*) and the dominant Bran, which has already been analyzed (see p. 356). There was, further, sooty, which, however, was lost and therefore is dubious. In one of the five identical  $F_1$  the dominant Bran appeared in a strange way, discussed above (p. 360).  $F_2$  from this (see pedigree, table 146) had in one brood 2 dachs ♀ among 72, also one ♂ with abnormal abdomen which was irregularly inherited and could not be extracted, and 1 gynandromorph. The last-named must have had a genetical basis, since one sister brood also contained a gynander and a third one appeared in a later generation—altogether too many for a chance result. In addition, one sister brood had 9 sooty ♀ among 34 which turned out to be *e*<sup>+</sup>. In one  $F_2$  with a rather unusual sex ratio, bred from one of the gynanders, dwarfs (2 ♂ among 76) appeared which inherited the character irregularly. In  $F_2$  of the parallel cross, dachs also segregated in two broods unrelated to the former. Further, 3 ♂ among 24 were bran blistered, which contains both another bran allele (*bran*<sup>2</sup>) and a *poi* allele *poi sq* (isolated from this cross).

In  $F_4$  of the first line in an outcross designed to test the Bran-*poi bl* combination (phenotype dumpy blist) among the segregants of this type (4966, 13 ♀ 10 ♂) there were 4 ♀ with rough eyes and bristles somewhat resembling forked. They were mated to soft blist brothers and produced Bld and soft blist offspring; the next generation contained 9 such ♀ (no ♂) among 75 Bld or soft blist. (Another  $F_4$  from 4966 contained a single such ♀ among many ♀.) They were mated for analysis, but did not



TABLE 146

## PEDIGREES

F <sub>1</sub> 3922 poi blist × Bld All daughters Bran Bld ( <i>dom. Bran</i> )	3736 poi bl × Bld
F <sub>2</sub> 4512 Bran Bld × poi 2 ♀ <i>dachs</i> among 72; 1 ♂ <i>abn. abd.</i> ; 1 <i>gynander</i> ; 1 ♂ <i>abn. wings</i> 4539 Same cross, 9 ♀ <i>society</i> among 34 4511 Same cross, 3 more F <sub>2</sub> 4511 Same cross, 1 <i>Gynander</i>	3736 <sup>2</sup> ♀ ♂ Bld. ♂ poi and (2) Df
F <sub>3</sub> 13 F <sub>2</sub> and test crosses 4698 = 4512 <sup>2</sup> Gyn × Bran 2 ♂ <i>dwarf</i> 1 poi <i>abn abd.</i> ; 20 ♀ : 76 ♂	3966 Bld × Bld 3 ♂ among 24 <i>bran<sup>2</sup> bran<sup>2</sup>, poi<sup>2</sup></i> 3973 Same cross 21 ♀ 17 ♂ <i>dachs</i> with Bld or 3976 Bld × poi , poi among 75 ♀ 96 ♂ 3 more F <sub>3</sub>
F <sub>4</sub> 4966 Bld × soft bl 4 ♀ among 51 <i>rough etc (M)</i> 4 more F <sub>4</sub>	7 F <sub>4</sub> and test crosses
F <sub>5</sub> 5101 (soft bl × r) <sup>2</sup> 8 ♀ <i>poi:dp</i> among 74 = <i>bran<sup>4</sup>p</i> 5103 Same cross, 1 ♀ <i>poi:dp</i> among 88 5 F <sub>5</sub> and tests	4814 = (Y × soft bl) <sup>2</sup> 1 ♀ <i>snipped</i> (?) 3 more F <sub>5</sub> <i>Many dwarfs</i>
F <sub>6</sub> 5180 (Y × 5102) <sup>2</sup> 5162 Same cross, <i>triplo-X</i> 3 more F <sub>6</sub> and tests	9 F <sub>6</sub> and test crosses 5031 = 4813 <sup>2</sup> dwarf <i>dachs</i> × <i>dachs</i> 1 ♀ 8 ♂ <i>slender</i> = <i>bran<sup>1</sup>/bran<sup>1</sup>, poi<sup>2</sup></i> among 14 ♀ 18 ♂
F <sub>7</sub> 3 F <sub>7</sub>	16 F <sub>7</sub> and tests
F <sub>8</sub> 5294 = 5208 <sup>2</sup> poi:dp 1 <i>Gynander</i>	5189 = 4813 <sup>4</sup> 1 ♂ <i>Bideroid</i> and <i>dachs</i> among 62 7 more F <sub>8</sub> and tests
F <sub>9</sub> 5329 = 5294 <sup>2</sup> poi × poi 2 ♀ N among 78 ( <i>Notch Df</i> ) 1 more F <sub>9</sub> (sister)	14 F <sub>9</sub> and tests
F <sub>10</sub> 1 F <sub>10</sub>	4 F <sub>10</sub> and tests
F <sub>11</sub> 5378 out of poi:dp 23 ♀ 5 ♂ with <i>bran<sup>2</sup></i> 5 more F <sub>11</sub> and tests	7 F <sub>11</sub> and tests

reproduce their kind any more. They were, obviously, hardly viable Minutes. In F<sub>5</sub> of the parallel cross (4819) 1 ♀ with extremely scalloped wings—resembling Df(2)vg—and rough eyes was found among many. She was sterile.

In F<sub>4</sub>, *dachs* had been crossed again with the stock Cy/d. In the offspring dwarfs appeared which were inherited rather irregularly. A stock could be established. In

F<sub>3</sub>, when dachs segregated again, 1 ♂ dachs among 68 ♀ 62 ♂ had scalloped wings. From it a stock of a new dominant sex-linked mutant, Beadexoid, was derived. Another F<sub>4</sub> ♀ of the first line was tested by breeding to a rudimentary ♂ also containing *svr<sup>poi</sup>* (from stock *r<sup>px bi</sup>*). In one F<sub>5</sub>, 8 among 74 ♀ were *poi : dp*, which we already found to be a specific combination of *bran<sup>ap</sup>* with *svr<sup>poi</sup>*, i.e., *bran* had mutated to *bran<sup>ap</sup>*. In another F<sub>5</sub>, 1 ♀ among 87 showed the same type. In the sixth generation an unusual result was found. Soft blistered males of the extreme rudimentary-like type were crossed to an attached-X female, with the usual result (♀  $\underline{y}$  ♂ *poi*). Twice an F<sub>2</sub> of such a cross contained triplo-X females (not yellow, but also not normal in color, scalloped, rough-eyed, sterile). Once only, 3 among many females (but, once, half of a huge number of females) survived. The soft blist ♂ must have contained something which made triplo-X females viable (the chromosomes were checked in the ovary and found to be triplo-X). As this was not the case in numerous similar crosses, some mutant must have been present. Actually, in one case a  $\underline{y}$  sister of the triplo-X females produced in the next generation again triplo-X daughters, and so did further generations as well as repetitions of the crosses. Once more the same thing happened when a soft blist and dwarf ♂ of the same origin as the former cases was crossed to  $\underline{y}$ . Already in F<sub>1</sub>, 1 triplo-X ♀ was obtained, and in F<sub>2</sub>, 6 among 41! We did not succeed in isolating the condition responsible for survival of triplo-X, which must have been autosomal. Again, in F<sub>3</sub> bred from pointed flies, 2 notched females among 78 appeared and turned out to be an N deficiency. From the lines containing both N deficiency and *poi : dp* the new allele *bran'* was obtained and isolated out of *poi : dp* parents. In the other line in F<sub>3</sub> a brood from dachs and dwarf parents produced, in addition to the expected types, 1 ♀ 8 ♂ slender, which means mutation of *bran* to *bran'* (or of + to *bran'*, *bran* not always being distinguishable with dachs), and from + to *poi''*.

Thus, in this line of eleven generations, after crossing *poi blist* and *Blond* and selecting out *Bld* after four generations, the tested mutants *Bran*, *bran'*, *bran''*, *bran<sup>ap</sup>*, *bran'*, *poi''*, *poi'*, *dachs*, *dwarf*, *Beadexoid*, *N-Df*, and *sooty* were isolated; and the probable but not isolated mutants *abnorm 4966 (M)*, "snipped," triplo-X enhancer, gynander-enhancer, abnormal abdomen were encountered, i.e., probably 17 (certainly 11) mutants among about 15,000 individuals. (An exact number cannot be given, because a great number of broods were test crosses for segregating or new types in which normal or expected results were simply marked without counts.) The pedigree (table 146) shows the two parallel lines with indication of only the first appearance of the mutants. As these appeared in direct lineage of the original cross as well as in offspring of outcrosses made to test segregating types (the series was made for the analysis of *poi blist*), the broods of each generation are not itemized except in the case of mutation.

One of the most frequent mutants derived from the different *poi* alleles in inbreeding as well as in outcrosses is contained in the phenotype one wing pointed, one wing dumpy (see p. 373), this *poi : dp* being a combination of *svr<sup>poi</sup>* with *bran<sup>ap</sup>*. The latter, then, is the frequent mutant, recognizable only in this combination. One of the stocks was isolated from flies reported in the foregoing pedigree. (For data on this see pp. 373 ff.) Among thousands of flies in this stock, occasionally one or a few soft blistered flies appear (never obtained thus far in pair breeding). During the analysis of this situation (see p. 379), which we may consider as a continuation

In the former pedigree, more facts belonging to the present section were found. Soft blistered in this case is produced by a mutation of *bran*<sup>u</sup> into *bran* or *bran*<sup>r</sup>. The pedigree, then, started with *bran*<sup>u</sup>/*bran*<sup>r</sup>, *poi* flies. In *F*<sub>1</sub> from *poi* : *dp* / *poi*, 10 out of 27 ♀ were *N*-deficiency, which was combined with *poi* and *bran*<sup>u</sup>. There were only 10 males, i.e., about  $\frac{3}{4}$  of the males missing. The conspicuous deficiency, which could not have been overlooked in former generations, thus must have arisen in half (or more) of the eggs of the mother, which was *bran*<sup>u</sup>, *bran*<sup>r</sup>, *poi* / *poi*. In each generation the main types were tested with the markers *bw e ey*, without special results. One *F*<sub>2</sub> was obtained by backcrossing a special type which had segregated (with arched opaque wings) to a male of *poi* : *dp* stock. One female and male each of the *poi* : *dp* type were tested against the marker stock. In both cases all daughters were sooty, rather dark; *e*<sup>+</sup> had changed its dominance, very pronouncedly in the females, less so in the males. This dominance-enhancer was inherited to the next generation and could be recombined as an autosomal dominant with homozygous *e*. Such flies were extremely black, showing that the action of the enhancer was more than that of an intensifier for ebony. In the same generation one brood was extracted from *F*<sub>2</sub> parents *bran*<sup>r</sup>/*bran*<sup>r</sup>, *poi* / *poi* × *bran*<sup>r</sup>/*bran*<sup>u</sup>, *poi* (phenotype: rudimentary blistered varying to soft blistered). It bred true, in accordance with expectation, but one male *poi* : *dp* appeared, i.e., a return mutation from *bran*<sup>r</sup> to *bran*<sup>u</sup>. Again, in the next generation *F*<sub>4</sub> from (*poi* : *dp* × *bw e ey*)<sup>+</sup>, one of those containing the new ebony enhancer. 2 among 87 ♂ were *bran*, which means that *bran*<sup>u</sup> had mutated into a higher *bran* allele. One more doubtful mutant, arched opaque wings, segregated in a few individuals from different generations and was linked with the second-chromosome marker. After breeding true it completely disappeared from an extracted stock and might have been a chance modification, not a mutation. Finally, in a further outcross involving this arc opaque type 1 ♀ giant among 28 appeared (heritable).

One more characteristic case may be mentioned. A pointed blistered female (= *bran bran*; *svr*<sup>poi bi</sup>) was mated to lanceolate speck (11 sp), and *F*<sub>1</sub> males backcrossed to pointed blistered. It turned out that the 11 sp father had been heterozygous for a second-chromosome dominant, lethal when homozygous, which produces shortened bristles of a bobbed or achete phenotype and very variable expression. Hence in the backcross the pointed flies showed this bristle character and the *poi* / *blist* (= *bran bran*) flies did not. Three out of 100 males, all with the intact pointed chromosome, were forked, which bred true as a forked allele with reduced fertility and an irregular heterozygous effect which had an interaction with the second-chromosome bristle character in so far as females heterozygous in both had short, stiff, stubble-like bristles. Later the second-chromosome character was transferred by crossing over into the *bran* chromosome, as shown by *poi* / *blist* and short-bristled flies, and segregation of 11 sp, which could not segregate before because of the lethal dominant. It is quite possible that the forked mutant had nothing to do with the presence of the dominant, though the coincidence is remarkable. Two generations later, *svr*<sup>poi bi</sup> mutated back to normal in a chromosome containing the new forked. The mother had been *svr*<sup>poi bi</sup> f / *svr*<sup>poi bi</sup>, the father *svr*<sup>poi bi</sup> f. The offspring segregated into 31 ♀ 32 ♂ pointed f and 28 ♀ 38 ♂ forked, but 7 ♀, i.e.,  $\frac{1}{8}$  of all ♀, were not *poi*, likewise 21 ♂, i.e.,  $\frac{1}{4}$  of all males. One of these males was a dwarf. All the flies tested were sterile, so that a further analysis was excluded. Again, a few gen-

erations later, in a large brood all *poi* blist and half of the males forked, bred from *poi* blist flies with heterozygous *f*, there appeared 5 yellow males, 1 without, 3 with forked, and 1 with abnormal abdomen. Pointed is not visible with yellow, but since the *poi* blist character was present it had not changed; the combination, with and without forked, must have been due to crossing over *poi-f*, which shows that the mutation had occurred in an early ovogonium. All yellow flies were sterile; hence we do not know what happened at this locus. Again we find interrelations which are not clear but are hardly based on pure chance. Nothing could be found in the forked region of the salivaries.

These examples of the type of happenings may suffice. These and some other such series are summarized in table 147, which shows that in these cases about one mutant in 1,000 flies was found,  $\frac{1}{4}$  of them at the *bran* and *poi* loci and about  $\frac{1}{2}$  dominants. This of course is not a quantitative result, since the experiments were not planned for this purpose, and the pedigrees used for table 147 were selected because they showed the high rate of mutation. It should be noted, however, that although they are not fit for a measurement of mutation rate, they are certainly highly significant in themselves.

The same argumentation applies to the following data. A great many individual mutational steps were found in  $F_1$  or  $F_2$  of crosses with *px bl* and derivatives, but there were many more such crosses which did not yield mutants. Again, the positive results are significant for our problem without being quantitative.<sup>14</sup> Table 148 contains the data. Among the mutants contained in this and the other tables only one suggests that it had been present in the tester stock in heterozygous condition, namely, *dachs*. This segregated three times after crossing with Blond translocation in  $F_2$  or  $F_3$ , once in two individual  $F_2$ . If *d* is present in the translocated chromosome it remains balanced in the stock, but may come out after crossing via crossover (*d* is far away from *Bld* in the second chromosome). In this case it might also segregate the same way in surviving *Bld*(1)*Df* females which have the two not *Bld* chromosomes. It is remarkable that occasional individuals appear with slightly bowed legs, like beginning *dachs*, though I never found *dachs*. This low type, which I recorded as half *dachs*, segregated also in these *Bld* crosses, either alone or together with real *dachs*; in the first case the real *dachs* appeared after a few generations. The low *dachs* neither bred true nor produced *dachs* in a compound with standard *dachs*. It looks like a low allele with extremely low expressibility. The real *dachs*, however, was never obtained in other numerous outcrosses of *Bld*, including a large control series, backcrossing ten pairs over and over to Canton wild. Thus it looks as if the real *dachs* from *Bld* were another allele produced in the crosses with *poi* and *bran* from the low allele sometimes present in *Bld*. (The real *dachs* bred true, though it is highly infertile, and gave a *dachs* compound with standard *dachs*.) It may be significant that *dachs* was obtained twice in other crosses than with *Bld*.

In many cases the chromosome which had mutated after crossing could not be assigned to those of the original parents, for lack of markers. But sometimes it was possible. Thus the *N-Df* in 2 ♀ among 74 in table 148, no. 13, occurred in the *ec br* chromosome after crossing to a male containing *poi* in the *X* and *ro op* (another mutant) in the second chromosome. Such facts seem rather significant.

<sup>14</sup> Quantitative experiments were started as soon as the progress of this work permitted. Sickness twice caused their abandonment. They have been started again.

TABLE 147  
MUTATION AFTER CROSSING

No.	Cross	Gen.	No. broods	Number		Mutants			
				Flies	Mut.	Certain		Probable	poi, bran, px, ls±±
						Not isolated	Isolated		
1	poi* × bran blist.....	3	30	3,251	2	1 poi suppr.....	.....	1 M.....	1 bran→poi
2	slender × bran blist .....	4	12	± 800	2	1 modif. for soft bl	.....	1 y curled	1 poi bl→plus
3	poi blist × ll sp.....	10	29	± 3,000	3	1 y.....	1 f.....	.....	.....
4	slender × poi.....	6	9	± 700	2	1 shaven depillate	1 giant.....	.....	.....
5	slender × a px sp .....	3	5	± 500	2	.....	.....	1 M; 1 dwarf.....	.....
6	y-Inv × Bld.....	10	83	7,037	5	D.....	sple, 3 alleles 4 times.....	.....	.....
7	poi bl × Bld a.....	11	140	±15,000	10	M <sub>1</sub> .....	N, d.....	Gyn.; abn. abd; dwarf; triplo-X-mod.....	Bran, bran <sup>dp</sup> , bran <sup>r</sup> poi <sup>ac</sup> , poi <sup>s</sup> , bran <sup>s</sup> , bran <sup>l</sup>
8	poi bl × Bld b.....	11	..	.....	7	.....	d, dwarf, Bldexoid	snipped.....	bran <sup>dp</sup> return, bran <sup>r</sup>
9	poi:dp from 8 (soft bl).....	8	74	± 4,500	6	c-enhancer, giant.	N.....	ap. arch? .....	.....

TABLE 148  
MUTATION AFTER OUTCROSSING OF px bl AND DERIVATIVES

No.	Mutant	Cross	Numbers	Remarks
1	dachs (d) two alleles.....	(bran × Bld) <sup>2</sup> and ff.....	5 ♀ 6 ♂ low d among 68 ♀ 105 ♂ F <sub>2</sub> .....	In later generations high-grade d is found; see text
2	Bd (Beaded).....	F <sub>1</sub> bb <sup>+</sup> /CIB × poi achi.....	3 ♀ among 80.....	Is Bd allele
3	ro op and bran <sup>dp</sup> .....	F <sub>2</sub> (svr <sup>poi</sup> × svr <sup>poi</sup> h) <sup>2</sup> .....	Few ♀ ♂ among many (1/8).....	
4	bran <sup>dp</sup> .....	F <sub>1</sub> svr <sup>poi</sup> × svr <sup>poi</sup> h.....	1 ♀ among many.....	
5	bran <sup>dp</sup> , Bx.....	Stock 5145 poi (lish).....	2 ♀ poi:dp, 1 ♂ Bx.....	
6	bran <sup>+</sup> , Bx low allele.....	(Patt × 6317 achi) <sup>2</sup> .....	1 ♂ Bx, bran <sup>+</sup> segregates next generation.....	
7	b <sup>3</sup> (dom. black).....	F <sub>1</sub> px bl × bs.....	Few ♀.....	
8	dp (dumpy).....	(px bl × X <sup>9</sup> ) <sup>2</sup> .....	1 ♂.....	
9	bran, poi.....	(y × px bl) <sup>2</sup> .....	1 ♂ soft bl among 309.....	px bl contained bg <sup>ap</sup>
10	poi → +.....	(w poi × poi:dp) <sup>2</sup> .....	1 ♂ among 52.....	
11	px.....	soft bl × (SD × soft bl).....	1 ♂ among many.....	
12	vesiculated (vs) and l.....	ro op poi × +.....	1/2 ♂ missing, others vs.....	
13	N-Df.....	(ec br × ro op) <sup>2</sup> .....	2 ♀ among 74.....	
14	L Lobe.....	(px bl × o wo tx) × o wo tx.....	26 ♀ 13 ♂ among 78 ♀ 88 ♂.....	New allele
15	L Lobe.....	(soft, x poi) <sup>2</sup> .....	5 ♀ 2 ♂ among 44 ♀ 30 ♂.....	
16	o allele.....	Stock soft bl 5085.....	1 ♀.....	Phenotype different from soft blist but genetically same
17	M.....	poi × Bld.....	A few.....	Sox-linked M sterile
18	e, L.....	(px bl × bw sp ba) × bw sp ba.....	2 ♀ e, 1 ♀ 1 ♂ L among only 8 ♀ 10 ♂.....	L sterile
19	dp-vo.....	(poi bl × X <sup>9</sup> ) <sup>2</sup> × px ec br.....	14 ♀ 4 ♂ among 95 ♀ 41 ♂.....	
20	d-dachs.....	(px bl × ec ei g) <sup>2</sup> .....	3 ♀ among 82.....	Sterile
21	abn. abd.....	px bl × (px bl × bs).....	.....	Abnormal abdomen and male genitals, sterile
22	bran <sup>+</sup> , giant, dwarf.....	bs × (px bl × F <sub>1</sub> (a) and F <sub>1</sub> .....	.....	
23	bran <sup>+</sup> , giant.....	(px bl × Ore) <sup>2</sup> and F <sub>4</sub> .....	.....	
24	bran <sup>dp</sup> , dwarf.....	(poi × Fx) <sup>2</sup> .....	.....	
25	M (3).....	poi × Bld.....	1 ♂.....	See list of mutants
26	bran <sup>dp</sup> , poi → +.....	(poi × lb) <sup>2</sup> .....	1 ♀.....	
27	bran <sup>dp</sup> .....	(poi × a px sp) <sup>2</sup> .....	16 ♀ among 150 in 4 broods.....	
28	bran <sup>dp</sup> .....	(poi × I (1) y <sup>px</sup> a px <sup>+</sup> +) <sup>2</sup> .....	1 ♀ among 79.....	
29	dp-vo.....	(poi:dp × bran) <sup>2</sup> .....	1 ♀ among 64.....	
30	bran <sup>+</sup> .....	poi:dp × bran.....	1 ♀ 3 ♂ among 110 ♀ 60 ♂.....	

In the foregoing tables a large number of apparent mutants were not included, because they defied analysis, though the type of their appearance strongly suggested "mutants," or in fact rarely surviving small duplications or deficiencies from small translocations. This interpretation is based upon the reappearance of some of these dubious mutants in many instances, their usual sterility, and also their appearance as a considerable number of individuals from crosses involving *px bl* and derivatives. The most frequent types are the dwarfs and yellowish dwarfs. Two hereditary types of dwarfs will be mentioned below; the others either were sterile or did not reproduce their type. To the same category belong types with short bristles or with missing posterior dorsocentrals, with rough eyes or small eyes, and with asymmetrical types of wings (1 wing strap-like, etc.) or eyes (1 eye small or missing) or antennae (- antenna without arista), etc. Innumerable such individuals were mated. The majority were sterile, which makes me believe that a genetic basis is to be assumed; the fertile ones did not reproduce their kind, which might mean that it was only a modification, but might also mean that a deficiency or duplication was almost unviable and only the normal segregants survived. Another phenotype of this category is abnormal abdomen. The occasional appearance of such individuals is a typical feature; they probably are not hereditary. But in a number of cases specific types of abnormal abdomen appeared in segregating broods, in up to one-fourth of the individuals, all of which had the same type: Once, all had defective not coneresced tergites along the entire middle line of the abdomen; once, all abdominal segments alternated in all individuals; once, all abnormal females were without the last two abdominal segments (not retracted genitals but real loss of segments). In no case could the hereditary character be proved. But thinking of Blond translocation where the very characteristic (1)Df type may segregate in considerable numbers and again be completely absent in a series of simultaneous crosses, I am inclined to assume that in these cases also the segregating types are rarely surviving duplication deficiencies from small translocations which might be located only if by chance known loci were involved in the cross.

I am encouraged in this belief because once by chance such an occurrence was traced. A cross (no. 591) of *px bl* × *yw* produced 71 ♀ 58 ♂ + and one white female. Both this exceptional female and her brothers and sisters were checked and shown to contain neither a white deficiency nor a mutation to white in the *px bl* X chromosome (see p. 467). A test cross, bred from normal  $F_2$  females with stock *yw* males showed some of these females to be normal, some heterozygous for + and *yw*, and one for *w* and +; further tests showed the latter to have been produced by crossing over between *y* and *w*. But in this cross an unusual array of abnormalities appeared with respect to eyes, wings, bristles, and size, as table 127 has shown. Most of these abnormal individuals were sterile. Whereas the X chromosome introduced originally from *px bl* turned out to be completely normal so far as the *w* locus was concerned, the exceptional white female must have been the product of changed dominance. In the following generations of test crosses the abnormal types nicked wings, small eye (also many abnormal abdomen, once 8 among 30 ♂), again appeared and were sterile. Part of the solution was found when white ♀ and ♂ extracted from no. 1032 in table 127 were crossed and backcrossed with X *ple* (9 recessive markers including yellow and apricot *w*\*). It turned out that in some of these crosses part of the segregating X\* males did not show any yellow; further,

that all crossover classes showed yellow, and all the reciprocal classes without yellow were missing. This indicates that from the original X chromosome the tip containing yellow, not white, had been broken off and translocated. The dominance effect of w found in the first instance was clearly a so-called position effect of the type of the cubitus interruptus effect, produced by a break near w. This was demonstrated in the  $w^*/w$  compounds produced in these crosses, which usually were more or less apricot but, in the presence of the translocation, white. Unfortunately, an enforced interruption prevented the salivary analysis, and the translocation was meanwhile lost (other details have already been reported, p. 467).

Among the many mutants at the *svr* and *a* loci there is one which in all cases of its origin seems to be connected with a definite rearrangement simultaneously present but not identical with the mutant. We have described above the type slender which is a combination product of *svr*<sup>po1</sup> and the *bran* allele *bran*<sup>1</sup>, the former producing soft wings (in addition to pale color), the latter having not much of a phenotypic effect if alone. The following is the story of the origin of slender.

A cross was made of a plexus blistered ♀ to a Florida ♂ (no. 665), with the intention of testing the crossover situation at the *px* and *bs* loci, and also the reciprocal cross (no. 668).  $F_1$  was in accordance with expectation. Some  $F_1$  flies were crossed to different second-chromosome stocks without unusual results.  $F_1$  females were also backcrossed to *bs* males, likewise without unusual results. Two backcrosses of the *bs* females with reciprocal  $F_1$  males (plexus blistered × Florida and Florida × plexus blistered) both gave unexpected results. It should be mentioned that the *bs* stock from Pasadena had bred true for generations and that numerous simultaneous crosses between the plexus blistered and the *bs* line had not yielded anything unexpected with the exception of one line, reported already, which had produced the dominant black-Deficiency (see table 143). These two backcrosses yielded:

- 1) No. 912 ♀ *bs* × (*px bl* × *Flor*). 38 ♀, 22 ♂ all giants.

Hatching of flies (at 25°) started only on the 19th day. The flies were extremely large and withstood ether for at least five times as long as normal flies. Their other characters (venation) were as expected in this cross. I mention that giants had once before been produced from dwarfs in the plexus blistered line, which makes a mutation in the Florida or *bs* stock improbable. The giant character was obviously dominant, though none of the parents were giants.

- 2) No. 913 *bs* × (*Flor* × *px bl*). 39 ♀, 25 ♂ of the different expected types of venation, all giants as before. Breeding of giants from these broods resulted in the following  $F_2$ :

No. 1143 = 913<sup>a</sup>

4 ♀ 3 ♂ giants partly short bristles, one with small eyes

57 ♀ 38 ♂ all grades from normal size to dwarfs (not classifiable)

No. 1146 = 912<sup>a</sup>

20 ♀ .. ♂ giants

68 ♀ 21 ♂ intermediates

10 ♀ 10 ♂ dwarfs

No. 1147 ditto

5 ♀ .. ♂ giants

42 ♀ 44 ♂ more or less intermediate

4 ♀ 5 ♂ dwarfs

No. 1148 ditto

9 ♀ .. ♂ giants

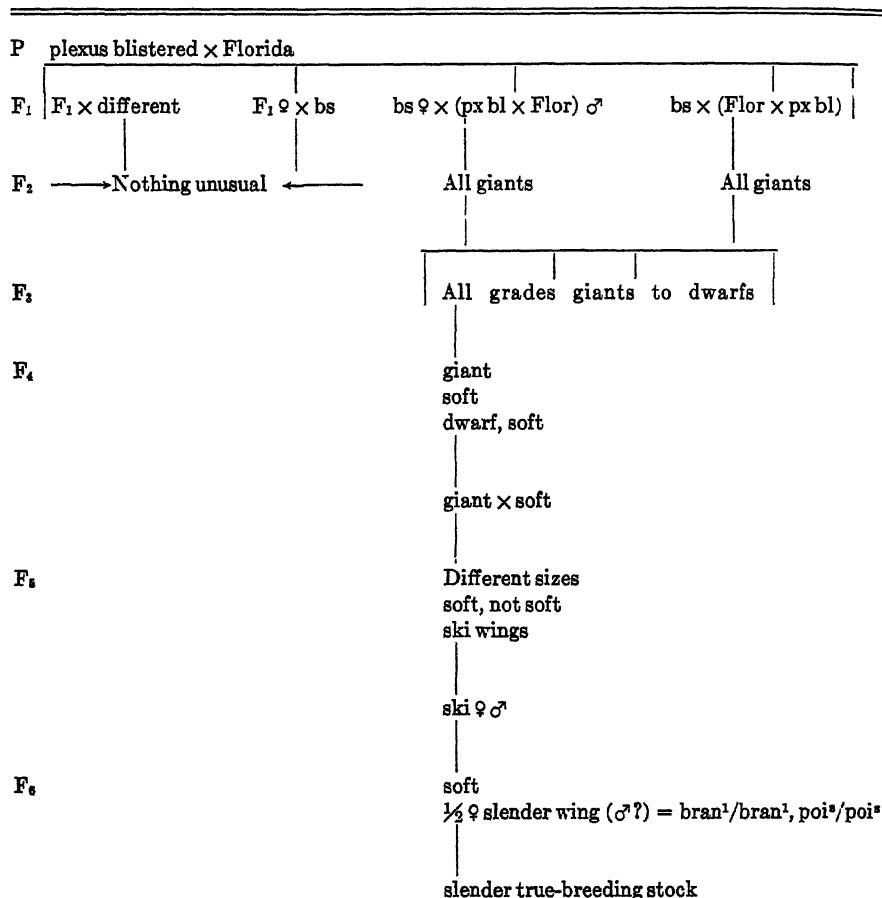
80 ♀ 44 ♂ all grades intermediate to dwarf (not classifiable)

Most of the dwarfs had soft wings, i.e., the X chromosome from *px bl* with the mutant *svr*<sup>po1</sup>. The dwarfs and giants were tested in subsequent generations. A



giant line with a changing percentage of giants has been established, but a dwarf line could not be bred. One of the  $F_4$  generations bred from intermediate-sized flies from 1147 (see above) contained 72 giant females (a few blistered) with normal venation, 12 males +, 18 males soft wings (males not giant); another similar one contained 25 ♀ giants, 2 ♀ with abnormal wings (unequal length, short), both being

TABLE 149  
PEDIGREE OF slender = bran<sup>1</sup> + poi<sup>s</sup>



sterile, ♂ soft, 1 dwarf and soft. From the latter brood an  $F_6$  was bred from the giant ♀♀ and soft ♂♂. It contained flies of different sizes, soft and not soft, and in addition (no. 1363) 2 ♀ 3 ♂ with ski wings. All these ski flies were mated and turned out to be rather infertile (see the low fertility in the foregoing generation). They produced altogether 30 individuals, females and males, part of the males with soft wings and half of the females with a new type of wing, narrow and slender, called slender (some of the males had the same wings, as tests showed, but hidden because of soft). From this brood (1410) one combination of slender females with soft wings produced all slender offspring, which since bred true (1436). Another brood gave

some slender offspring and a majority difficult to classify. The ski flies, then, had been heterozygous for *bran*<sup>1</sup>, which obviously had arisen by mutation somehow connected with the giants and dwarfs in the presence of *svr*<sup>poi</sup> derived from *px bl*. The pedigree table 149 shows the general features of the case.

On a later occasion the mutant *bran*<sup>1</sup> was again produced in connection with giants. In *F*<sub>2</sub> from *px bl* × *Ore*, giants were found, and also soft was present in the *X* derived from *px bl*. A giant ♀ with a soft ♂ (no. 2297) produced one-half normals (very large but not giants) and one-half ♀ and ♂ slender. No dwarfs were found, but it is possible that they were not viable and that actually only  $\frac{1}{4}$  slender segregated. Slender bred true and turned out to be the *bran*<sup>1</sup>/*bran*<sup>1</sup>, *poi s* combination as before.

Once again slender was obtained (see pedigree, p. 367) in a way which required a mutation from *poi bl* to *poi s* and in addition from *bran* to *bran*<sup>1</sup>. In this case both parents were dwarfs but no giants had been recorded.

Once, the opposite relation was observed. Among a great many outcrosses involving slender there was one, (slender × *bw e ey*)<sup>2</sup> no. 2053, in which a large number of giants segregated. Slender thus may still contain the ancestral condition which produces giants, or be able to reproduce it.

The interest thus centers upon the giants and dwarfs. Giants and dwarfs have repeatedly been found in *Drosophila*. Some of them behaved like ordinary mutants, but some offered difficulties to an analysis in addition to the variability of penetrance and expressivity (literature quoted in *DIS* 16). The most complicated case is that of Bridges's giant, which is stated to be based upon an unknown dominant, homozygous lethal in the second chromosome, in conjunction with a homozygous recessive near locus 64 in the third chromosome. The males, moreover, are sterile. But in none of these cases has a relation of giants to dwarfs been asserted. Such a relation, so far as I know, has been described only for the duplication deficiencies segregating from reciprocal translocations in Blond and pale, i.e., one of the combinations being giant while the opposite combination of duplication and deficiency is dwarfish. We expected, therefore, some such situation in our case. But neither translocation nor deficiency tests corroborated such an assumption and the salivaries seem to be normal, though one or two band alterations, difficult to find if the region is not known, cannot be excluded. The main facts found are the following:

1. From the pedigree reported above, a giant stock could be established. It breeds true, though the penetrance is very variable and real giants are rather rare in ordinary conditions, but increase, strangely enough, in old bottles. Male giants are rarer than female ones but are not sterile. Dwarfs are never segregated within this stock.

2. Apart from the cases mentioned, only once was a true-breeding giant isolated. It reverted to normal in the bottles after many generations. It seems that this was a different giant, identical with Bridges's giant 4 (2.24) as indicated by a localization test made before it reverted.

3. It is remarkable that giants (which could not be extracted) have also been obtained in the so-called *achi* lines, containing *svr*<sup>poi</sup> and a high *bb* allele. Bridges-Gabritchevsky's giant also appeared in conjunction with a *bb* allele.

4. Dwarfs are the most frequent occurrences both in pure stocks of *px bl* and its derivatives and in all kinds of outcrosses.

5. These dwarfs have been tested innumerable times. They produced usually normal offspring in *F*<sub>1</sub>-*F*<sub>2</sub>.

6. Repeatedly, dwarfs appearing after the manner of mutants produced large, very large, or even giant offspring in *F*<sub>1</sub> and all transitions from giants or very large flies to real dwarfs in *F*<sub>2</sub>. No true-breeding dwarfs could be extracted.

7. Only once (see pedigree of forked, p. 508) could dwarfs be isolated as a sex-linked mutant which had appeared together with forked and a *bb* allele. No giants were involved in this case.

8. When dwarfs segregated in  $F_2$  out of dwarf grandparents and giant  $F_1$ , their numbers were extremely small.

9. While the extracted giant stock threw dwarfs, giants and dwarfs segregated in  $F_2$  after out-crossing the stock.

10. These  $F_2$  suggest a rather complicated situation which might be related to, though not identical, with Bridges's 2-3 giant. The following table (table 150) contains the results of  $F_2$  giant  $\times$  bw, e, ey)<sup>a</sup> only for the females. Out of 24 identical  $F_2$ , 4 contained only flies without size differences; the others segregated giants, intermediates, and dwarfs, with the exception of 3 broods without giants. The table contains the total numbers for all classes with the expectations for ordinary  $F_2$  recombinations in all groups, as giants and dwarfs were present in all classes of the markers.

TABLE 150  
(giant  $\times$  bw, e, ey)<sup>a</sup> ♀ ♀

	Class							
	+	bw	e	ey	bw e	bw ey	e ey	bw e ey
Expectation for markers . . . . .	27	9	9	9	3	3	3	1
giants : exp. markers . . . . .	153	45	45	45	15	15	15	5
giants : number . . . . .	171	29	69	16	11	14	6	4
intermed. : exp. markers . . . . .	535	178	178	178	59	59	59	20
intermed. : numbers . . . . .	638	204	210	59	49	21	32	7
dwarfs : exp. markers . . . . .	81	27	27	27	9	9	9	3
dwarfs : numbers . . . . .	67	53	24	5	23	7	6	4
Total : exp. markers . . . . .	751	250	250	250	83	83	83	28
Total : number . . . . .	921	286	303	80	83	42	46	15

The table shows in all groups the repeatedly discussed suppression of eyeless, which does not require further comment. There is no marker class without giants or dwarfs. But there is a clear reciprocal relation between the bw and e classes: among the giants there are 29 bw, 69 e, and among the dwarfs 53 bw, 24 e, the intermediates and the totals containing equal classes. This statistically significant difference can be interpreted as meaning that giants require the homozygous second chromosome from giant stock and dwarfs the third, the smaller number present in the two classes respectively being crossovers.

The ratios of giant : intermediate : dwarf in each class ought to be instructive. There is reason to believe that giants and intermediates cannot be clearly separated, though the error should be always of the same magnitude. But the counts of dwarfs are rather reliable. Looking at these ratios, we find the number of dwarfs much smaller than that of giants in all sufficiently large classes with the exception of bw and bw e; in bw there are 29 giants and 53 dwarfs, and in bw e 11 giants and 23 dwarfs as opposed to 17 : 67, 69 : 27, 16 : 5, 14 : 7 in the other classes. In the classes with many dwarfs the ratio not dwarf : dwarf is  $293 : 76 = 3.9 : 1$ ; in the other classes,  $1198 : 106 = 11.3 : 1$ . This might mean that dwarf segregates as a simple recessive when the second chromosome from giant stock is absent, but in its presence is suppressed or lethal, maybe only in the homozygote.

The complicated situation indicated by these data is apparent also when the males are considered. In 9  $F_2$  broods in which the females behaved as reported, the males

did not show any size differences. In this case the sex ratio was  $917 : 670 = 1.37$  and significantly differed from 1:1. In 10 broods the males were classifiable. In only two of them could giants be distinguished, which, however, may be due to poor expressivity. But dwarfs segregated clearly. In this group the sex ratio was  $1018 : 924 = 1.1 : 1$ . The distribution of the dwarf males in the classes was:

Class	+	bw	e	ey	bw e	bw ey	e ey	bw e ey
Not dwarf : dwarf	434 : 47	111 : 47	93 : 31	33 : 7	22 : 17	8 : 5	31 : 5	19 : 7

This is a very different distribution from that found for females, the only clear high ratio being found in the absence of homozygous foreign chromosomes (the + class). No interpretation can be offered. But the sex ratios clearly indicate some participation of the X chromosome. Whereas the sex ratio is normal when dwarfs segregate, but one-fourth males are missing (sex ratio 4 : 3) when no dwarfs segregate, it is probable that in one combination of X chromosome and one autosome the males are lethal instead of dwarfs. Whereas the sex ratio is normal when the cross is made, so that all males have the X from giant stock, namely, ( $y$ , bw, e, ey  $\times$  giant)<sup>2</sup>, it follows that the foreign X (not from giant stock) is responsible for the lethal class.

It is remarkable that one of the rather frequent mutants, namely, bran<sup>ap</sup>, recognized in the combination with poi called poi : dp (see p. 373), also seems to be connected somehow with dwarfism, as the following pedigree of one instance of its origin demonstrates. Test crosses for the analysis of the bs locus in poi were made. In two of them, nos. 5506 and 5507 ( $svr^{po1} \times bs$ )  $\times$  ( $svr^{po1} \times Px$ ) and ( $svr^{po1} \times Px$ )<sup>2</sup>, one and two females of the wing type between pointed and dumpy, frequently occurring in the poi : dp combination, were found together with one male poi and dwarf (among 147 ♀ 146 ♂). The two not virginal mutant females were mated to their dwarf brother. Among about 150 daughters of different types (the mother had been prefertilized), 6 females of the maternal type and short bristles and one female with dumpy-like wings appeared. All had much plexation and therefore must have contained Px. A sister brood, also from not virginal mutant females, contained only one dumpy-like male, again with much plexation, and one male dwarf. Offspring of not mutant sisters were normal. Again the mutant females were not virginal when found and therefore were bred as mass culture. In the offspring, 3 ♀ 1 ♂ of the original mutant type appeared and 6 ♀ of the typical poi : dp features. From these a poi : dp line was extracted. The mutant bran<sup>ap</sup> thus had appeared originally in a few gametes of both F<sub>1</sub> parents, while dwarfs were also produced. No more information about the coincidence is available.

Once more a similar relation was found, this time in connection with a return mutation in the a-px region of the second chromosome. We have described above (see p. 498) how in a px bl stock in which the y-Inv had originated the px mutant was lost by some reversion to normal. When these normals were extracted, their offspring contained (no. 3949) 4 ♀ 6 ♂ dwarf among 146 flies. Their offspring were very large though not giant. In a later generation dwarfs again segregated, but no rule could be established.

## 5. SUMMARY OF MUTANTS AND THEIR ORIGIN

In table 151 we have summarized the occurrence of mutation and return mutation at the px, bs, svr, and a loci as reported in the paper. This table contains by no

TABLE 151  
FREQUENCY OF MUTANTS AT MAIN LOCI IN ALL RECORDED CASES

Mutant	In controlled crosses and checked				Found in mass culture of		Return mutation	Remarks	Σ In pair culture	Σ In mass culture
	From px bl	From svr alleles	From crosses within derivatives of px bl	From outcrosses	px bl	poi				
svred.....	1	..	1	4	....	....	4, many times in bottle.....	Many times in outcrosses; not checked further. ....	10	Many
svred <sup>h</sup> ....	..	..	..	..	Many times	....	.....	Only once from heat-treated Oregon.....	1	
svred <sup>h</sup> ....	..	1 from poi	..	2	Many times	....	.....	Most frequent mutant within px bl stock.	3	Many
svred <sup>h</sup> ....	1	..	..	1	....	....	.....	.....	1	
svred <sup>h</sup> ....	1	1	2	..	Repeatedly	....	1 → poi.....	.....	5	Repeatedly
svred <sup>h</sup> ....	1	..	1	..	....	....	.....	.....	1	
svred <sup>h</sup> ....	1	1	2	1, many more	....	Many times	.....	Both cases in presence of poi and bb.....	2	
bran <sup>1</sup> ....	..	..	..	5	....	....	.....	.....	5	Many
bran <sup>1</sup> ....	..	..	..	1	....	....	.....	.....	1	
bran <sup>1</sup> ....	2	..	1	..	....	....	.....	.....	3	
bran <sup>1</sup> ....	1	..	1	..	....	....	.....	.....	2	
bran <sup>h</sup> ....	..	1	..	..	....	....	.....	.....	1	
bran <sup>h</sup> ....	..	2	1, 1 from bran	5	....	....	1.....	.....	1	
bran <sup>h</sup> ....	..	..	1	2	....	....	.....	Many times more in all groups; most frequent mutant ..	10	A great many times
bran <sup>h</sup> ....	..	..	..	2	....	....	.....	Frequently from bran <sup>h</sup> .....	3	Frequently
brp.....	..	..	..	..	....	....	2 and in bottles repeatedly.....	.....	2	
brp <sup>h</sup> ....	1	..	1	..	....	....	Repeatedly in bottle.....	Repeatedly in stock .....	2	Repeatedly
px.....	..	..	1	1	....	Repeatedly	5 and repeatedly in bottles.....	.....	7	Repeatedly

means all instances, but, in the main columns, only those in which the new mutants or the reversions have been extracted and tested. It is certain that some of them have occurred much more frequently (see last column), especially *poi*, *poi s*, and the *poi:dp* combination. Single individuals of the last-named (the combination resembling the case of "mutable genes") have been found so frequently whenever *poi* was present in crosses that they were not checked any more for their identity,

TABLE 152  
SIMULTANEOUS MUTATION OBSERVED WITHIN ONE OR A FEW GENERATIONS INVOLVING  
SVT AND BRAN LOCI  
(Details in text)

No.	Simultaneous (or almost) mutants	Origin from	Remarks
1	<i>svr<sup>poi</sup></i> , <i>svr<sup>poi si</sup></i> , <i>svr<sup>poi bl</sup></i> , <i>bran</i> , <i>r</i> ; return high <i>px</i> → low, <i>bs</i> → +, <i>px</i> → +	<i>px bl</i>	
2	<i>svr<sup>poi</sup></i> , <i>bb</i>	Cross	
3	<i>svr<sup>poi</sup></i> , <i>suppr. bs ba</i>	Cross	
4	<i>bran</i> , <i>poi</i> → +	<i>poi</i> (many times)	
5	<i>bran</i> , <i>svr<sup>poi bl</sup></i>	<i>poi</i> × <i>poi h</i>	
6	<i>bran<sup>s</sup></i> , <i>poi<sup>sq</sup></i>	<i>poi blist</i> × Bld	
7	<i>bran<sup>i</sup></i> , <i>poi s</i> , <i>giant</i>	<i>px bl</i> × +	
8	<i>Bran</i> , <i>bran<sup>dp</sup></i> , <i>bran<sup>r</sup></i> , <i>N</i>	Bld × <i>poi blist</i>	
9	<i>bran<sup>dp</sup></i> , <i>Bx</i>	<i>poi bb</i>	
10	<i>bran</i> , <i>bran<sup>s</sup></i> , <i>bran<sup>i</sup></i> , <i>poi</i> , <i>poi bl</i> , <i>poi si</i> , <i>poi dish</i> , <i>bb</i> , <i>px</i> → +, <i>bs<sup>pp</sup></i> → +	<i>px bl</i> with <i>bs<sup>pp</sup></i>	
11	<i>poi<sup>dish</sup></i> from <i>poi</i> in presence of <i>a</i> , <i>bb</i> , <i>bs<sup>pp</sup></i>	<i>poi</i> × <i>a px sp</i>	
12	<i>poi bl</i> , <i>bran</i> , <i>bs<sup>pp</sup></i> , + → <i>px</i> , <i>poi</i> → +	<i>poi</i> × <i>poi h</i>	
13	<i>bs<sup>pp</sup></i> via notched	<i>px bl</i>	
14	<i>bran</i> , <i>poi</i> , <i>px</i> → +	<i>px bl I</i>	
15	<i>e</i> , <i>bran<sup>b</sup></i> , <i>poi</i> → +	soft <i>blist</i>	Not analyzed further
16	<i>poi s</i> , <i>bran</i>	<i>poi</i>	
17	<i>poi s</i> , <i>bran</i>	<i>px bl</i>	
18	<i>poi bl</i> , <i>bran</i> , <i>px</i> → +, + → <i>px</i> , spread	<i>I(1)Y<sup>px bl</sup> × px bl</i>	Not analyzed further
19	<i>bran<sup>i</sup></i> , <i>poi s</i> , <i>giant</i> , <i>dwarf</i>	<i>px bl</i> × +, twice. Once <i>giant</i> from slender.	

which in view of the characteristic phenotype is, however, not doubted. The transition from *poi* to *poi s* is probably just as frequent, but cannot be discovered easily if not combined with *bran*, and even then it may be overlooked without tests because the phenotype overlaps with selected extreme *poi*. Further, *bran* has mutated much more frequently than is shown in the table. In the presence of *px* it might be overlooked, and since the combination *px bran* is poorly viable and rather sterile, it was not always isolated. Later on, occasional *px* individuals resembling *bran* were not tested any more. Thus the contents of table 151 may be regarded as only a minimum.

At many points of the foregoing discussion we could have drawn attention to the fact that mutants appeared simultaneously or what appeared to be simultaneously. As such cases are of special importance, they are tabulated in table 152. This table shows how frequently *bran* and *poi* alleles appeared simultaneously by mutation,

though they also were found to mutate alone. Further to be noted are the correlation between mutation at these loci and return mutation at px and bs, and the occasional accumulation of mutation to different alleles, simultaneous mutation at other loci, and special coincidences such as dwarfs and giants in relation to the "slender" combination.

## LIST OF MUTANTS

*Abnormal abdomen.* A series of different hereditary types, none inherited in simple fashion or further analyzed.

**b<sup>B</sup>** *Black dominant* from cross plexus blistered  $\times$  bs.  $b^B/b$  is darker than  $b^B/1$  or e. Small deficiency. See text figure 4. This Df(21)35CD puts black farther to the right than in Bridges's map.

**Bd<sup>e</sup>** Allele of *Beaded* (3-93.8). Origin from cross  $bb^1/C1B \times 6340$  achi (= combination of  $svr^{poi}$ , a px sp,  $bb^{poi}$ , and Y chromosome with bb). A slightly higher allele than the original Bd, with the same characteristics (incomplete dominance, enhanced to full effect by Inversions in third chromosome, homozygous lethal).

**Bx<sup>61</sup>** Allele of *Beadex* (1-59.4). Origin 1 ♂ in offspring of pairs  $svr^{poi} d^{1ab}$  with bb (5145) together with bran<sup>6</sup>. Higher allele than standard Bx; otherwise same characteristics.

**Bx<sup>63</sup>** The same, but lower allele from  $F_2$   $y$  bw, e, ey  $\times$  6317 = poi,  $bb^{poi} h^1$ , and Y chromosome with  $bb^{poi} h^1$ .

**Bx<sup>65</sup>** The same not mentioned in text. Originated, after the tables were finished, from cross bran<sup>3</sup>,  $svr^{poi} e^1 \times$  Df(2)a px, as 2 males.

**Bxd** *Beadexoid* (1- $\pm$  45). Origin 1 ♂ in  $F_4$  from bran<sup>6</sup>,  $svr^{poi} h^1 \times$  Bld which was simultaneously dachs of earlier origin. Phenotype like a high-grade Beadex.

**bb<sup>poi</sup>** Low *bobbed* from pointed (1-66.0). Phenotype described in table 75. Origin repeatedly in first chromosome from  $svr^{poi}$  and other alleles (see special section on bb). A similar allele also found in plexus blistered stock.

**bb<sup>poi} h^1</sup>** The same, but higher allele; see table 75.

**bb<sup>a px sp</sup>** The same, but low allele in a px sp stock, brought out only after crossing to  $svr^{poi}$ .

**bb<sup>a px sp} h^1</sup>** The same, high allele. See table 75 and section on bb.

**bs<sup>p</sup>** *blistered<sup>p</sup>* (2-107.3). A slightly higher allele than bs present in px bl stock. See table of phenotypes 79.

**bs<sup>sp</sup>** The same, higher allele present in px bl stock and repeatedly produced by mutation. For origin see special section; for phenotype and compounds see table 79. Frequently associated with small deficiency right of ba.

**bran** *broad angular* (2-at or near arc). Probably arc allele (see special section). Originated many times from plexus blistered,  $svr^{poi}$  (see table 152). Wings broader, shorter, and blunt or broad at tip. At time of origin tendency to short bristles, later disappeared.

**bran alleles** bran<sup>1</sup>, bran<sup>2</sup>, bran<sup>3</sup>, bran<sup>4</sup>, bran<sup>ab</sup>, bran<sup>6</sup>, bran<sup>7</sup>, bran<sup>x</sup> (from X rays), Bran. Some phenotype like bran, some normal with effect visible only in presence of  $svr$  alleles. For origin see tables 151, 152. For phenotype and combination effect see table 74.

**curl** *curl* (2- $\pm$  60). Originated in stock  $y \times r$  from plexus blistered. Like low-grade Curly; characteristic combination effects with rudimentary.

**d** *dachs* (2-31.0). At least four different alleles, a low one with little expressivity, one like standard d, an extreme one, and a dominant one; the higher ones completely sterile. Repeated mutants after crossing. See table 148. Also originated in In(1) $y^{px} h^1$  stock in at least two alleles.

**Df(1)ec** *Echinus deficiency* in stock plexus blistered. Minute characters. Completely sterile females, homo- (hemi-)zygous lethal.

**dp** *dumpy* (2-13.0). Repeated, different alleles, lower like standard dumpy, higher with strong vortices. For origin see table 148.

**dwarf** Most frequent type, segregating into and from giants; only one could be isolated as sex-linked recessive. All others seem rare surviving Df-Dp from small translocations, though not proved. See special discussion, p. 513.

**e** *ebony* (3.70.7). Different alleles of a phenotype such as e<sup>a</sup>, and higher. For origin see table 148. **enhancer of ebony** (3- $\frac{1}{2}$ ). Once in a line of bran<sup>6</sup>,  $svr^{poi}$ . See table 148. Makes e<sup>a</sup>/+ darker than e<sup>a</sup>, and e<sup>a</sup>/e<sup>a</sup> darker than same with speck.

*f<sup>a</sup>*. A *forked* allele (1-56.7). Not completely like standard forked; tendency to loss of posterior scutellars; sometimes individuals of a stubble type. Origin as three males from bran, *svr<sup>poi</sup> b<sup>1</sup>* × (bran, *svr<sup>poi</sup> b<sup>1</sup>*), the latter containing a second-chromosome homozygous lethal Minute. Poor fertility.

*Giants*. Different occurrences, never simple mutants, usually related to dwarfs and probably segregating Df-Dp from translocations. See special discussion, p. 513.

*L<sup>62</sup>*. *Lobe* (2.72.0). Origin (plexus blistered × e wo tx) × e wo tx. Different from other alleles by variety of effects. All the usual types and, in addition, a tendency to increase eye into a short finger-like structure or to split off small parts of eye and dislocate over head. Frequently short palpi inside modified eye. Dominance not very considerable.

*L<sup>63</sup>*. *Lobe* from *svr<sup>poi</sup> \** × *svr<sup>poi</sup>*. Very similar to foregoing, if not identical with it.

*L<sup>64</sup>*. *Lobe* from (plexus blistered × bw sp ba) × bw sp ba. Like typical *Lobe*. Sterile.

*M. Minute*. Four different ones, one sex-linked, the others autosomal; not isolated or sterile.

*Modifier of dominance* of bran. Described in text; not isolated.

*Modifier of viability of triplo-X*. Makes triplo-X partly or completely viable; derived repeatedly from soft blistered but not isolated since the super-females are completely sterile; is autosomal.

*In(1)y<sup>px</sup> b<sup>1</sup>*. *Inversion yellow px bl*, spontaneous as one male in px bl stock. See picture of salivaries, text figure 3.

*op a. Opaque arched*. Second chromosome recessive from cross bran<sup>2</sup>, *svr<sup>poi</sup>* × bw e ey. Reverted to normal soon after establishment of stock. Wings opaque, cloudy, and arched.

*rop. Rough opaque*. (2d chromosome, probably different loci). Originated from cross *svr<sup>poi</sup>* × *svr<sup>poi</sup> h*. Small minute like but usually normal bristles. Short rounded wings. Rough eyes or not.

A formation at both attachments of posterior cross vein, tendency to doubling or tripling of anterior crossvein. Though true-breeding, difficult to recover after outcrossing.

*Snipped-like*. Rather exactly like Df(2)vg<sup>+</sup>. 1 ♀ from bran<sup>2</sup>, *svr<sup>poi</sup> b<sup>1</sup>* × Bld. See table 148. Sterile.

*Scalloped-like*. A low nicking of the wings with very little penetrance; discarded.

*sple. spiny legs* (2-± 55). Derived repeatedly from *In(1)y<sup>px</sup> b<sup>1</sup>* stock and outcrosses, as described in text. At least 3 alleles. Lowest type, tarsal hair of forelegs like spines or thorns and irregular; medium type, all legs of this type; highest types, all legs of this type and disheveled hair on body. It is possibly identical with ti tarsae (*sic!*) irregular of Ives; see DIS 16.

*su-poi*. Suppressor for pointed. Found in cross *svr<sup>poi</sup>* × bran<sup>2</sup>, *svr<sup>poi</sup> s<sup>q</sup>*, autosomal, not isolated.

*sv<sup>d</sup>*. Shaven depilate (4) from bran<sup>2</sup>, *svr<sup>poi</sup> \** × *svr<sup>poi</sup>*. Exactly like the description of this mutant, but completely sterile. Segregated in Mendelian ratios.

*svr<sup>poi</sup>*. *pointed svr* allele and the alleles *svr<sup>poi</sup> h*, *svr<sup>poi</sup> s*, *svr<sup>poi</sup> s<sup>q</sup>*, *svr<sup>poi</sup> b<sup>1</sup>*, *svr<sup>poi</sup> s<sup>1</sup>*, *svr<sup>poi</sup> disk*, as described in table 74.

*w. white*. White eyes obtained three times, checked for allelism to w and discarded. One white from X-raying is small inversion of sections 3CD.

*wD. Dominance enhancer for white*. See analysis, p. 468.

*y<sup>ca</sup>*. *yellow curled*. Pale yellow and slightly curled wings; from bran<sup>2</sup>, *svr<sup>poi</sup> \** × bran<sup>2</sup>, *svr<sup>poi</sup> s<sup>q</sup>*. Sterile.

*y'* *yellow*. Males as described with origin of forked; very light yellow. Completely sterile.

*y<sup>o</sup>* *yellow*. Yellow allele from extracted giant stock, not mentioned in text.

#### IV. TENTATIVE X-RAY EXPERIMENTS

Although this work deals with spontaneous mutation, a few strictly preliminary X-ray experiments were made. The intention was to see whether the mutation rate under radiation would be unusually high in the px bl stock and whether the typical bran and poi mutants would appear. For these experiments the stock *In(1)y<sup>px</sup> b<sup>1</sup>* was used, i.e., px bl in which the small yellow Inversion described above had originated (thus marking the first chromosome) and which had also been used for experiments in spontaneous mutation (see p. 498). Those experiments served as controls. The stock was first inbred in brother-sister cultures and simultaneously checked for variability. Only once in 180 bottles was a sex-linked lethal present in the mother. The degree of plexation and blistering fluctuated in the usual way. Occa-



sionally a few dwarfs appeared. Males were irradiated with about 4,500 r, 91½ cm. from tube. The irradiated males were mated to either y or Canton females.<sup>15</sup>

In a first small series only 10 irradiated males produced offspring. Out of 16 F<sub>1</sub>, 15 were normal, 1 contained 15 males among 117 with wings similar to bran and part blistered, both in y and not y classes. The type was inbred and tests showed that a new bran allele had arisen with definite features alone and in combination with poi, which are described in table 153.

Bran<sup>\*</sup> is thus more or less dominant. It resembles in many respects our bran<sup>2</sup> and bran<sup>ab</sup>. It is of interest that the yellow Inversion enhances the amount of blistering (best demonstrated in the cross y × bran<sup>\*</sup>, y-Inv). This is the same position effect of the right break of the yellow-Inversion already described (p. 399). (The presence of a poi allele was excluded.) Bran<sup>\*</sup> was never obtained in homozygous

TABLE 153  
PHENOTYPES OF X-RAY-PRODUCED bran<sup>\*</sup>

2d chromosome	1st chromosome	Phenotype
bran <sup>*</sup> .....	+... ..	Between bran and +, varying up to real bran (i.e., ± dominant). Tendency to blisters
bran <sup>*</sup> /+.....	y-Inv...	The presence of y-Inv increases tendency to blisters (most individuals are blist)
bran <sup>*</sup> /bran <sup>*</sup> ....	+ ..	Never obtained, probably because bran <sup>*</sup> is linked with px and lethal
bran <sup>*</sup> /bran....	+.....	Wings dumpoid (between bran and dp)
bran <sup>*</sup> /bran....	y-Inv.....	The same mostly blistered
bran <sup>*</sup> /+.....	poi.....	All transitions from pointed blistered to soft blistered and folded wings
bran <sup>*</sup> /bran....	poi.....	Rudimentary blistered

condition. It might be lethal *per se* as a kind of dominant. But bran<sup>\*</sup> had originated in a px-containing chromosome and was closely linked with this. Only one crossover px/px bran individual was obtained, but no bran without px. Since the bran px combinations are poorly viable and almost sterile, it is possible that this combination is responsible for the homozygous lethality and that, if separated, bran<sup>\*</sup> would be viable.

In view of a positive result with only 10 irradiated males, the experiment was repeated, and this time 25 fertile irradiated males were obtained. The results on mutation were very promising. Among four tests in crosses with y and involving only 18 X chromosomes, 1 w mutant was obtained which turned out to be a small inversion of sections 1, 3CD. (white had been obtained twice from px bl crosses, but in view of the frequency of this mutant this was not considered important and no salivaries had been studied). Further, among 20 crosses of irradiated males with Canton females, two different Notch deficiencies (in different crosses) and one Delta deficiency appeared among 520 females. Thirty-three of these females were bred to F<sub>1</sub>, and four contained a sex-linked lethal. An additional feature was the unusual array of abnormal individuals in F<sub>1</sub> of Canton × irradiated ♂. We know, of course, that X radiation can produce phenocopies in the irradiated individuals.

<sup>15</sup> Mr. R. E. Paulson kindly performed these irradiations coincidentally with his own work. I am greatly indebted to the Department of Genetics of the University of California, especially Dr. E. R. Dempster, for the use of apparatus.

But here numerous abnormalities appeared in the offspring. Among 520 ♀ 465 ♂ the following abnormal types were registered (we are not repeating the dominant mutants already reported):

♀ abnormal abdomen (1 contained a mutant)	♂ 2 blistered
1 gynandromorph	1 scute
1 abnormal abdomen and 1 wing bran	1 dwarf
1 short pointed wings	1 rough eye
1 one wing rudimentary; same side shorter abdomen, rough eye, 1 leg bent (not gynandromorph)	1 strange wing shape
1 one wing narrow, net, same side rough eye and shorter abdomen	1 low px (mutant)
1 crippled	1 one wing short
1 short bristles (contains a mutant)	
2 one wing short (1 mutant)	

Only two of these turned out to be heterozygous for a new mutant, namely, one female with a short wing was heterozygous for a new bran allele and one male low px was heterozygous for *bs*<sup>pp</sup>. Two more segregated mutants in later generations. Those with slight changes (scute, bobbed, abnormal abdomen) did not reproduce their character. The others were sterile. The frequent asymmetrical forms may be "mutants" with somatic segregation.

Further generations showed that more mutants had been produced. There was a bran allele, characterized by a little dominance and a tendency to blisters in the absence of *poi*, which was obtained in heterozygous condition in 4 males among 60, sons of the *F*<sub>1</sub> female with one wing shorter. This number is irrelevant because these 9 males were recognized by their dominance; other females and males probably were also heterozygous but not noticed. These males were crossed for testing to females with *y* and bran, and half of the offspring in both sexes were bran (part of the males blistered). The allele probably was bran<sup>2</sup>, if not the ordinary bran. In one of three *F*<sub>2</sub> from this test cross a few dachs females and males segregated, and in a further generation of one of the *F*<sub>2</sub> which did not contain dachs, 6 dachs among 92 were obtained. Dachs thus had arisen somehow in connection with the new bran but was very poorly viable and almost completely sterile. We had earlier noted many instances in which different dachs alleles had been obtained from *px bl* crosses.

From the *F*<sub>1</sub> male with low px, i.e., more plexation than the ordinary dominance effect of *px* and *bs* (derived from the *y px bl* male), an *F*<sub>2</sub> with a sister was obtained in which the typical combination of *px* with the allele *bs*<sup>pp</sup> (extreme *px bl* in both sexes) segregated. In a sister *F*<sub>2</sub> from normal parents 1 ♀ 1 ♂ of this type were obtained among a large number of the expected types. Thus *bs*<sup>pp</sup> had mutated to *bs*<sup>pp</sup> in a number of gametes of the irradiated *px bs*<sup>pp</sup> males (tested with *bs*).

In one *F*<sub>2</sub> bottle from normal *F*<sub>1</sub> a few Minute-like individuals segregated. They were bred to bran and turned out not to contain bran. In *F*<sub>2</sub> the type segregated again but was subsequently lost.

We have already mentioned the one *F*<sub>1</sub> female with abnormal abdomen (6019). It produced with a normal brother only 2 ♀ and 5 ♂. Both females and one male showed a kind of pointed form which a new mutant type called slender-dumpy was derived by outcrossing to *poi*, the first male having been heterozygous for this character. This new *poi* allele has most remarkable features and is accompanied by a

second-chromosome condition. Details will be presented separately. In a sister  $F_2$  from normal  $F_1$ , 1 ♀ 1 ♂ with broad and arched wings, probably a mutant, were obtained, both being sterile.

Finally, in the same  $F_1$  (6032) which contained one Notch female a short bristled female was bred to a normal brother. In  $F_2$ , among many normal flies one female was found with yellowish color, rough eyes, and opaque wings. She was mated to a brother and in the second generation a new type segregated which turned out to be a low allele of broad (br) in the first chromosome.

Thus, among 520 females and 465 males heterozygous for one irradiated autosome (only the females for the X chromosome), of which only 30 were tested, in  $F_2$ , in addition to the 2N and one  $\Delta$  found in  $F_1$ , there were produced 4 sex-linked lethals, at least 1 bran allele, at least 2 instances of  $bs^{pp}$ , 1 dachs, 1 Minute (?), the new slender-dumpy = a poi allele, and 1 broad (br). Not only is this an unusually high rate of mutation, but the regions are involved which are known to mutate in our line, namely, bran, svr, facet-notch, dachs, bs. Thus the preliminary results are rather encouraging, and special experiments will be conducted as soon as feasible.

## V. DISCUSSION

This paper presents data on spontaneous mutation and such facts concerning the genetic constitution of the mutating lines as could be found out. The general trend of these data is to suggest that mutation is not a haphazard event in a so-called gene molecule, but a phenomenon of a determinate, orderly type which is caused by conditions within the chromosomes. We prefer, at this time, to present primarily the factual data as they stand and not to enter into a full discussion of their bearing. We believe that such a discussion would be useful only if an attempt were made to analyze and correlate all the facts known about mutation and to confront them with our knowledge of the constitution of the hereditary material. We are confident that, even now, so exhaustive a discussion would cast serious doubt upon the reality of the so-called gene molecule; but in order to be convincing the discussion would have to be complete and elaborate, and not a short appendix to a factual paper. Hence, we propose only to mention such items from the foregoing analysis as, in our opinion, point away from the conception of chance happenings in a side chain of a gene molecule, and to indicate, not too specifically, in what direction a better solution for the problem of mutational changes within the chromosomes may be found.

The following groups of facts reported in this paper stand out as pointing toward the existence of certain rules governing the origin of spontaneous mutations.

In the main line and its descendants studied closely over a considerable time, definite loci show a tendency to mutate. This mutation produced repeatedly one and the same mutant, and frequently members of a allelic series of the same loci. Some of these alleles appeared frequently by mutation, others only once. The alleles include invisibles, recognizable only by combination effects; recessive and dominant alleles; one so-called unstable mutant (a second one was produced by X rays); and a few recognizable rearrangements. This applies to the arc and silver loci, both of which are otherwise normal in the salivaries, though remarkable features near by were described. Both of these allelic groups have a relatively high tendency to return mutation. The original stock contained mutants of px and bs. The latter

has also a tendency to mutate to a higher allele, and both rather frequently revert to normal. In normal descendants of reverted px, mutation to px can occur. In addition to these frequent mutants, other visible mutants are obtained from the original stock and its derivatives more frequently than usual after outcrossing. Most remarkable is the fact that, in rather small experiments with X radiation, mutation was produced at the same main loci (a and svr).

There is a remarkable tendency toward crowding of mutations. If, after crossing, a mutant appears, others will frequently be found in the same or subsequent generations. In a rather extreme way this is true for the mutants at the main loci. Reverse mutation of px and bs was repeatedly found simultaneously, though both loci mutated also independently. A very frequent occurrence was simultaneous mutation at the arc and silver loci, with respect to allelic mutants as well as reverse mutation. Again, mutation at these loci sometimes coincided with mutation at the px and bs loci, especially return mutation. Most probably there is also a correlation between mutations at the svr and bb loci.

In many cases, mutation was preceded by abnormalities which themselves were not inherited, e.g., scalloped wings, dwarfs, ski wings as parents of mutants which had nothing in common with these phenotypes.

The genetic analysis of the lines from which all these examples were taken revealed peculiarities which had to be interpreted as small nonreciprocal translocations and transpositions. Most of them involved the X chromosome and were therefore traceable through male lethal classes. Similar conditions in the autosomes not engendering known loci could hardly have been discovered. These abnormalities were present or absent in the individuals from nonselected populations, and, if present, some or all could be found. Only a part of them could be verified in the salivary chromosomes, involving only extremely small sections.

The salivary chromosomes of the lines studied showed a considerable array of small rearrangements, mostly of 1-3 bands, both deficiencies and translocations. Some of them were rather frequent, others rare, or very rare. Larger deficiencies, inversions, and translocations were very rare. This means the rearrangements above mentioned are present in the stocks without showing visible effects, and thus are in contrast to those rearrangements which have been connected with mutants. It is remarkable that a considerable number of these small rearrangements were identical not only in px bl, poi, and other alleles of the series derived directly or indirectly from px bl, but also in a completely independent mutant svr<sup>poi</sup> without any genetic relation to the other lines. But different wild-type lines were free from these small rearrangements. With the lone exception of the ec locus, none of them seemed to include known loci and none of them were located at the loci of mutants appearing in those lines (except bran<sup>2</sup>, which is a one-band deficiency). It is noteworthy that a considerable number of the small rearrangements are found in the neighborhood of the mutating loci, i.e., tip of X and right end of 2. But there is a suspicion that this is only evidence of closer scrutiny of the suspected regions. Finally, it ought to be said that, in two cases, mutation at the svr locus occurred in an X chromosome introduced into a cross from a tester stock, under exclusion of crossing over but in the presence of a bran allele. There is also one case in which, after X-raying sperm and crossing the X-rayed males, a mutant appeared in the nonirradiated chromosome at the svr locus. This requires an indirect causation of this mutant

by some unknown condition which had been produced in one of the irradiated chromosomes. The points mentioned before also suggest the probability of a causation of mutants at susceptible loci by conditions present or appearing at different loci. (See especially the coincident mutations and return mutations at the *a*, *svr*, *px*, *bs*, *bb* loci.) There are in the literature scattered observations of mutants which appeared some generations after X-raying or treatment with extreme temperatures.

Our own ideas in regard to the nature of mutation may be stated without detailed discussion in the form of the following working hypothesis. We start from the fact that the serial, polarized differentiation of the chromosome reveals the presence of a not very large number of sections (aside from heterochromatic sections) within which the different so-called point mutations are located and within which rearrangement breaks produce multiple alleles by the so-called position effects. The sections in question overlap under certain conditions (see discussion in Goldschmidt, 1944). The question has been frequently discussed (see literature quoted in former papers) whether it might be assumed that point mutations are small rearrangements. By definition a point mutation in *Drosophila* is assumed in the case of a mutant with normal salivary chromosomes in the decisive region and its immediate neighborhood. Thus we are confronted with the question, Is there a difference in principle between a mutant with a visible change in the chromosome and one without a visible change? As all other differences assumed to exist between both have broken down, we have to decide whether we shall dismiss the question because it is of no use to discuss what cannot be seen, or whether there are facts known which make such a discussion fruitful. I think that the latter is the case. Gottschersky (1937) studied a Notch deficiency with clear genetic results, i.e., deficiency for the facet and split loci; but the salivaries were normal. He offered the interpretation that the genes must have been inactivated, an explanation which is really only a confession that the theory of the gene does not furnish an interpretation. Later, Demerec (1941) found other similar cases in the same region. But meanwhile it was discovered (Kodani, 1942; Goldschmidt and Kodani, 1942) that a single band in the chromosome is not a unit but a coil of chromonema with 4-6 perultimate chromomeres radiating from it. There is therefore no reason to doubt that within a single band deficiencies or inversions of some of these chromomeres are possible. Could these be the "point mutations"? Thus far, technical difficulties have prevented a final answer. But we accept such a view as a promising hypothesis since it brings all mutations into line as mechanical disturbances of the serial order of the material which constitutes the decisive part of the chromosome.

Such an interpretation, we believe, would place all the facts of mutation, including those presented here, on the basis of mechanical happenings within the chromosome as opposed to chemical changes within a gene molecule. (I realize that "mechanical" may also mean chemical in the end, viz., if a break occurs between units of a polymerized chain and these are united again in a different way, new bonds must be established; "mechanical" as I use it thus means a change at a super-molecular level involving only primary bonds, not side chains.) I am inclined to visualize the facts of mutation in the following way.

The general facts of mutation require happenings which might occur in the chromosomes of any cell of the cycle, i.e., in any nucleus in resting condition or at mitosis. Not much is known about the condition of chromosomes in the resting

nucleus of diploid cells, except oocytes. The salivary gland and similar nuclei in the Diptera are the only clear examples. It is tacitly assumed that the completely synapsed condition of the resting chromosomes in these nuclei is a special feature of dipteran cells. I wonder whether this is not the typical condition of chromosomes in all resting nuclei? The behavior of the heterochromatin in resting nuclei, which seems to follow the pattern of behavior in the salivary-gland nuclei, may be cited in favor of such an assumption. This means that within the resting nucleus the condition of the chromosomes is such as to release the unknown attractive forces between homologues which produce the complete synapsis without chiasmata, as in the dipteran nuclei. In mitotic prophase this attraction ceases, maybe in conjunction with the growth of the split halves of each chromosome. (There are a number of data in older cytological literature describing tetrad-like structures in the prophase of ordinary mitosis.) Only in the Diptera is this attraction still visible in metaphase. The only other example known to me occurs in the division of cells of parthenogenetic frogs (see Goldschmidt, 1920). But in the latter case the homologues are the product of a secondary regulation in an originally haploid set, which might be a different situation. If the chromosomes are synapsed in the resting nucleus, the presence of a number of small translocations, etc., would produce the well-known abnormal synaptic configurations. These in turn would lead to stresses and torsions in the chromosome which might result in breakage, pulling out of small sections, reinsertion. If the chromosomes in the resting nucleus are completely stretched (including molecular unfolding), as might reasonably be supposed, the same breaks might occur within what corresponds to a salivary band. The result is the production of small invisible rearrangements and so-called point mutations.<sup>36</sup> I can visualize the possibility that the knowledge of the presence of small rearrangements at definite points of the chromosomes would permit a prediction of the sections in which new mutants are most likely to occur because of maximum stress. All the facts presented in this paper, as well as as many observations of other authors on some kind of controlled mutation, agree with an explanation of the type just presented, and at the same time create serious difficulties for the hypothesis that the gene molecule changes its side chains in mutation. Quantitative experiments intended to corroborate our viewpoint were started repeatedly, but each time had to be broken off, owing to unfortunate external circumstances, before they had reached sufficiently large numbers. We hope to finish them and to return afterward to a more detailed discussion of our conclusions, including a review of known facts which are in harmony with our viewpoint.

## VI. SUMMARY

1. The work is based upon a stock and its derivations, namely, plexus blistered. Px bl is derived from standard px by mutations at the bs locus, together with the rise of different modifiers for plexation and blistering.

2. The starting point was a spontaneous set of simultaneous mutations in a controlled brood of known pedigree which included return mutation from px and bs to normal, and mutation at the arc, silver, and rudimentary loci.

<sup>36</sup> Though I am refraining here from a complete discussion of the literature, I might mention that recently Fano (Proc. Nat. Acad. Sci. Washington 29, 1943) pointed out that stresses along chromosomes and interchromosomal pulls influence the rejoining of broken ends.

3. A similar "upheaval" occurred twice again later in mass culture, producing the same return mutations and different alleles at the *bs*, *svr*, and *a* loci.

4. Mutation at the same loci, including the return mutations from *bs* and *px* to +, recurred repeatedly in controlled broods, some more, some less frequently.

5. This includes the mutation to a series of multiple alleles at the *svr* and *a* loci, and repeated mutation at the *bs* and *bb* loci; it includes also secondary return mutation from + to *px*, return mutations of the *svr* and *a* mutants to +.

6. Each of these steps was found to occur individually, but in a significantly high number of cases two or more mutations or return mutations (including mutation from one allele to another) occurred simultaneously, pointing to some interrelation.

7. In many cases abnormal sex ratios coincided with these occurrences. Detailed accounts relating to points 5-7 of a number of cases are given.

8. Though the mutation rate within *px bl* and its derivatives is very low, mutation occurs very frequently after crossing between these stocks, or after outcrossing. In this case, too, a tendency to an accumulation of mutants was observed, i.e., when one appeared, others were found, usually within a few generations, including alleles at the main loci already mentioned. Some of these (visible) mutants were found repeatedly and also in different alleles, e.g., *Lobe*, *Notch*, *ebony*, *dachs*. Some such cases are presented in detail, others are only tabulated. Though quantitative experiments could not be finished on a sufficiently large scale, the data are unequivocal.

9. Rather frequently, abnormal individuals were found which were sterile or which transmitted their character to only a small part of their offspring. In some cases these became the parents of completely different mutants.

10. There are many additional details in connection with the foregoing points, e.g., strange ratios of segregation, as well as sex ratios, and sublethality of homozygous *bran* when present together with *px*.

11. Many individual cases of simultaneous mutation suggest a relation of the type found in translocations with more or less viable deficiency-duplication classes with typical phenotypes, as in *Bld* or *P*.

12. In a small set of tentative X-ray experiments with this stock an unusually high percentage of mutants was obtained, and among them also the ones at the *silver*, *arc*, *blistered*, *dachs*, and *Notch* loci, known to mutate spontaneously.

13. The genetic analysis of *px bl* and the derived *silver* stocks revealed the presence of a number of special features present in addition to the already mentioned mutants at the *px*, *bs*, *svr* loci, X chromosome modifiers responsible for blistering in the presence of *px* and *bs*<sup>9</sup>, other modifiers for the degree of plexation, and others for the expressivity of the pointed wing character of *svr*<sup>10</sup>.

14. The most typical of these features are nonreciprocal translocations of small sections, not containing known loci, from the X chromosome—near the left end—to the second near *arc* and to the third right of *ebony*, which are recognized by the presence of male deficient lethal classes. In addition, the data indicate a transposition of the same sections from the left end of the X chromosome, one to the right between *v* and *wy*, the other to near the right end.

15. These insertions were already present in the *px bl* stock—none, or one or more, or all of them simultaneously in different individuals—and are typical for the *svr* mutants. There seemed to be some relation between these conditions and the mutation at the *svr* and *a* loci.

16. It is remarkable that many of the same rearrangements can be present in an independent *svr* allele derived from Florida stock.

17. Some but not all of these rearrangements could be located in the salivary-gland chromosomes.

18. In the silver stocks as well as in *px bl* from which they originated the second and (or) third chromosomes contain suppressors for the expression of *eyeless* of different degree of action. In some alleles a dominance enhancer for *eyeless* was found with special features which were not completely understood.

19. The salivary chromosomes of *px bl* and its derivatives contain a large number of very small rearrangements, some of which are found in every individual, others being more or less rare. They were studied statistically. Larger inversions and translocations were extremely rare.

20. In the most frequent mutants, the *svr* alleles, the *svr* locus is always normal. But in a number of the alleles a typical disturbance is found 6 bands to the right. In one allele it is a 2-band inversion. In others the typical aspect had been interpreted as a 1-band inversion. In still others an interpretation of the minute disturbance was not possible.

21. At the *arc* locus only one of the mutants was a 1-band deficiency; all others had normal chromosomes. Among the other mutants were some visible deficiencies, but most of them had normal chromosomes.

22. Many other interesting features are found in the *svr* alleles, sometimes different in alleles of different origin, viz., dominance-enhancing actions on *arc* and *ebony*, suppressor action of *bs* and *ba*. The former actions have an autosomal basis, the latter are located in the X chromosome.

23. The different *svr* alleles have different phenotypic effects upon different characters, i.e., pointed wings, soft wings, suppression of speck and sable, and recombination effects with mutants at the *arc* locus. They are compared with the effects of other loci in the same region, namely, Blond translocation and the color suppressors. A discussion of all facts and their meaning for the understanding of small chromosome sections is presented.

24. The combination effects of mutants at the *svr* and *a* loci (including deficiencies) are studied. They are of interest for the understanding of epistasis and the phenogenetics of wing shape, which is discussed.

25. One of the *svr* alleles behaves like one of the so-called mutable genes. Its genetics is analyzed and interpreted without recourse to mutable genes. (Another such case involving the same locus has been produced by X rays and will be presented separately.)

26. Among the combination effects of *arc* and *svr* loci are those in which an inversion or translocation break in the *svr* region acts like an allele of *svr*. In one of these cases a small inversion is involved with the left break in the yellow region and a yellow position effect. Thus the effects at both breaks could be demonstrated. There is a discussion of this set of facts.

27. As the work was done with the *px bl* stock and its descendants, we tried to get as much information as possible about the genetic composition of this stock. The main data have already been summarized. The analysis required detailed study of phenotypes of compounds, which gave a number of results not directly connected with the main line of work, namely:



*a.* An analysis of wing plexation as produced by the second chromosome mutants *px*, *bs* and alleles, *ba*, *net*, *Px*, their compounds and combinations.

*b.* Data on special behavior of balloon and net, further on some new *bs* mutants.

*c.* Data on blistering as produced by the loci mentioned as well as by modifiers in the X chromosome.

*d.* Phenogenetic consideration of wing plexation.

*e.* An analysis of some relations between the actions of bobbed alleles derived from *px bl* homozygotes and the different action in compounds upon bristles and abdominal tergites.

28. A considerable number of incidental observations are scattered through the report, e.g., data on *Bld* translocation, on surviving of triplo-X, on blistering in a wild-type stock, on *ec-Df* in *px bl*, a Dubinin effect for the *w* locus, different dominance enhancing or suppressing combinations, and others.

29. A short discussion presents the author's conclusions on mutation as not entailing a chemical change in a gene molecule but mechanical disturbances of the serial order of the material which constitutes the decisive part of the chromosome, including those below the level of one salivary band.

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# PLATES

## PLATE 23

Wing plexation of a few different types in illustration of the description of phenotypes in table 79.

Fig. 1. The compound *bs/bs*<sup>9</sup> with "antler" near posterior cross vein.

Fig. 2. The compound *bs/bs*<sup>99</sup> with "web" in the same location.

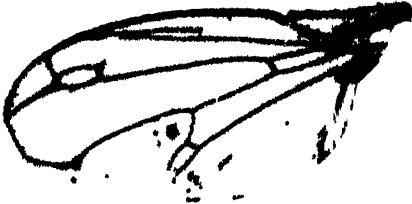
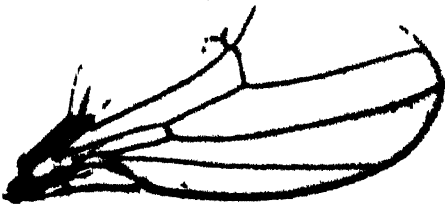
Fig. 3. Lower type of *px bl* with plexation in I and II, E.V. in V, antlers at 2, 3, 4, and web near cross vein. (The two wings are not completely symmetrical.)

Fig. 4. Higher type of *px bl*, with complete parallel and periclinal veins (in right wings), antler or web at 2, 3, and large web connecting parallel and periclinal veins.

Fig. 5. Plexation of the mutant net with the large web at the posterior wing edge into which the ends of 4 and 5 merge.



1



3



4



5



6

## PLATE 24

Salivary chromosomes in the test series of *poi*, *poi h*, and *px bl* as summarized in table 45, 46, 129. M. Kodani del.

Fig. 1. Df(1)1C<sup>1</sup>, 1-3 from *px bl*. See table 129, no. 2.

Fig. 2. Df(1)3D, 1-4 from *px bl*. See table 129, no. 5.

Fig. 3. Df(2L)26, C1 from *px bl*, not in main series.

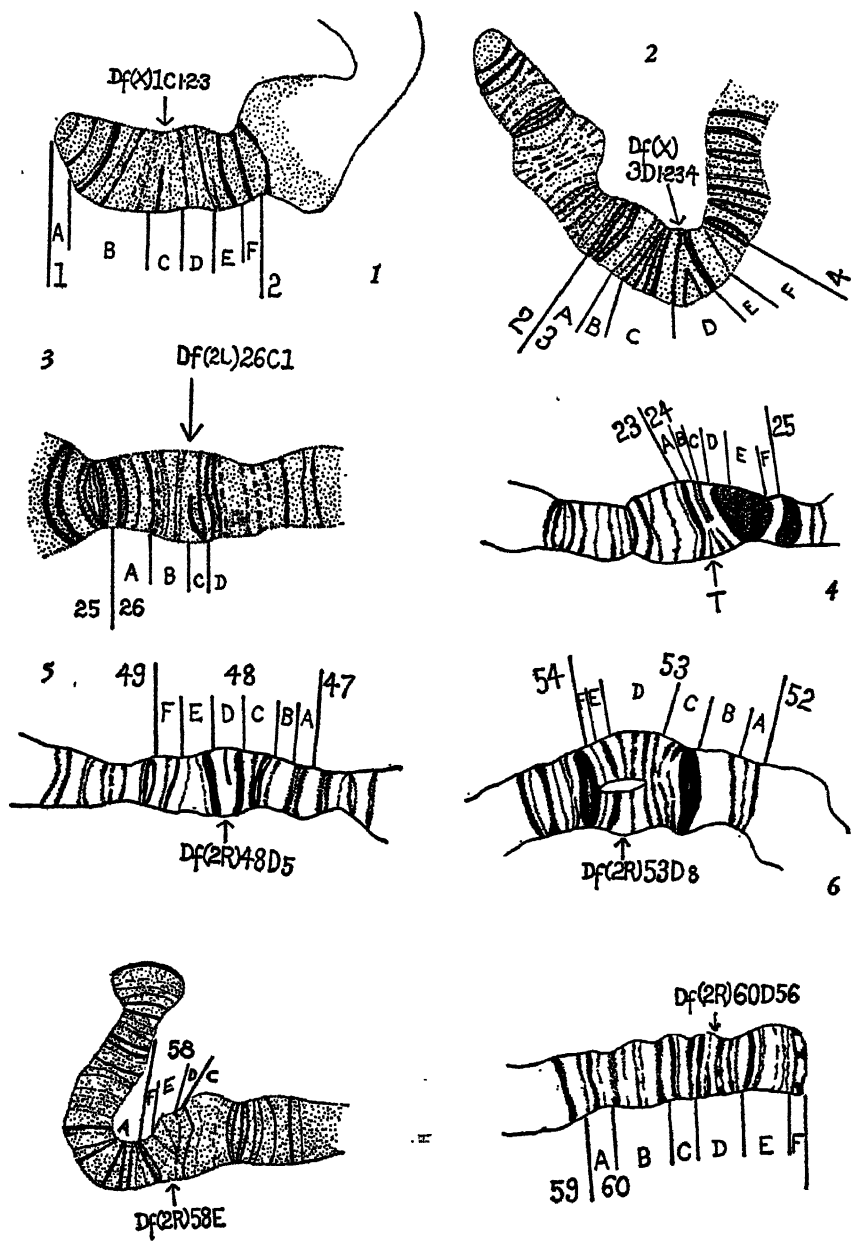
Fig. 4. T(2L)24, AB from *poi*. See tables 46, no. 5; 45, no. 10; 129, no. 10.

Fig. 5. Df(2R)48D, 5 from *poi h*. See table 45, no. 6.

Fig. 6. Df(2R)53D, 8 from *poi*. See table 45, no. 7.

Fig. 7. Df(2R)58E from *poi*. See tables 46, no. 7; 129, no. 12.

Fig. 8. Df(2R)60D, 5, 6 from *px bl*. See tables 46, no. 8; 129, no. 13.





## PLATE 25

Salivary chromosomes in the test series of poi, poi h, and px bl as summarized in tables 45, 46, 129. M. Kodani del.

- Fig. 1. Df(1)9A, 2, 3 from poi. See tables 46, no. 3, 45, no. 4, 129, no. 7.  
Fig. 2 Df(1)9A, 2, 3 from poi See tables 46, no. 3, 45, no. 4, 129, no. 7.  
Fig. 3. Df(1)3A, 2 from px bl. See table 129, no. 2.  
Fig. 4. Df(1)4C, 9 and 4D, 1, 2 from poi. See tables 45, no. 3; 129, no. 6.  
Fig. 5. Df(1)4C, 9 and 4D, 1, 2 from poi See tables 45, no. 3, 129, no. 6  
Fig. 6. Df(1)D5 from poi, not from series  
Fig. 7. T(1) right of 3C, 7 from poi. See tables 45, 46, no. 2.  
Fig. 8. Df(1)9F, 13 from px bl. See table 129, no. 8.  
Fig. 9. T between (1)12E, 8 and 9 from poi. See tables 46, no. 4; 45, no. 5.

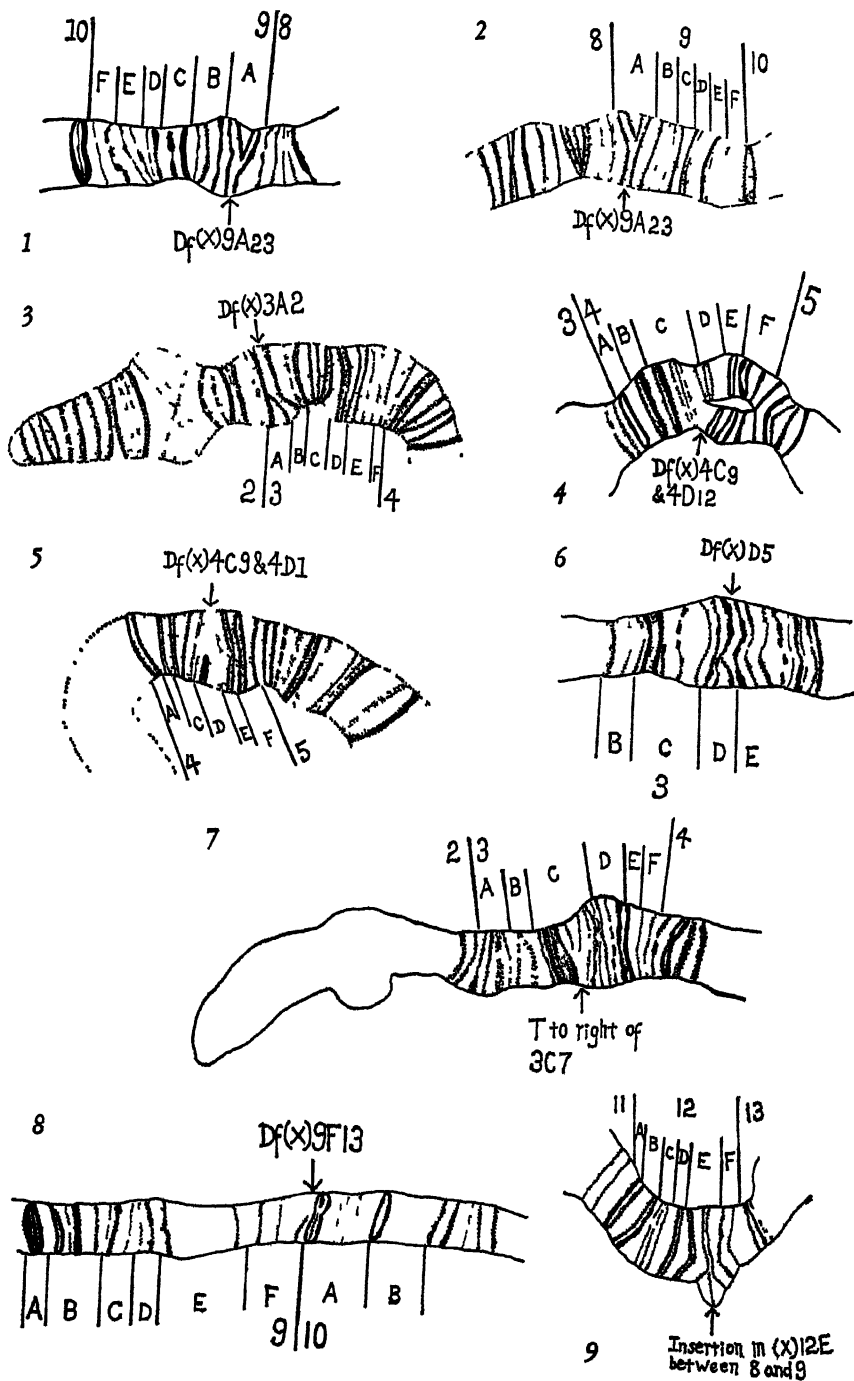


PLATE 26

Salivary chromosomes in the test series of poi, poi h, and px bl as summarized in tables 45, 46, 129. M. Kodani del.

Fig. 1. Df(2R)58D, 5 from px bl. See tables 46, no. 6; 45, no. 8, 129, no. 11.

Fig. 2. Df(3L)61C, 8 from poi. See tables 46, no. 11, 45, no. 12; 129, no. 16.

Fig. 3. Df(3R)91C, 4, 5 from poi. See tables 46, no. 15; 45, no. 16.

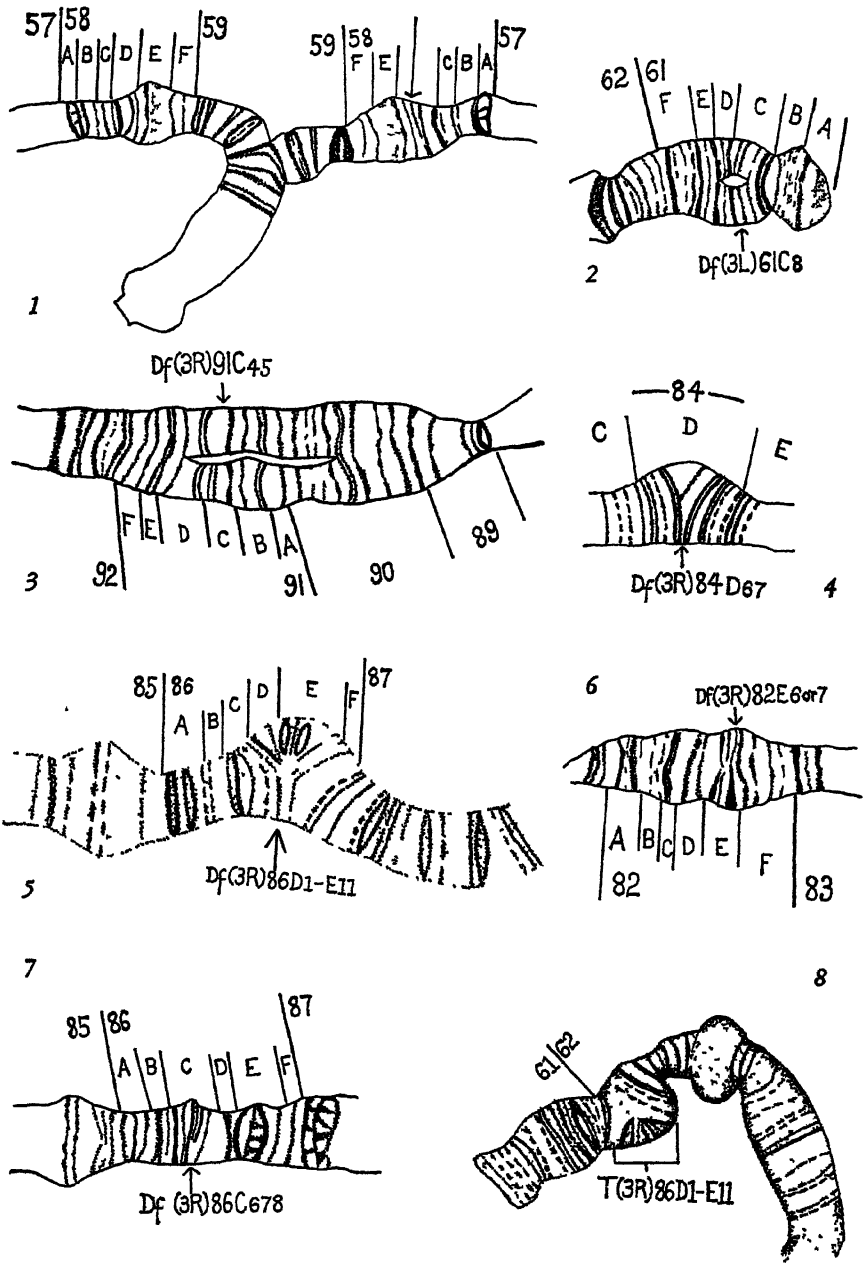
Fig. 4. Df(3R)84D, 6, 7 from poi. See tables 46, no. 13; 45, no. 14; 129, no. 19.

Fig. 5. Not from main series; Df(3R)86D, 1-E, 11 translocated into 3L, 61, 62, shown in figure 8.

Fig. 6. Df(3R) 82E, 6 (7?) from homozygous poi; not main series.

Fig. 7. Df(3R)86C, 6-8 from poi. See tables 46, no. 14; 45, no. 13; 129, no. 21.

Fig. 8. See figure 5.



## PLATE 27

Salivary chromosomes in the test series of poi, poi h, and px bl as summarized in tables 45, 46, 129 M Kodani del

Fig 1 T to tip of 4 from px bl See table 129, no 23

Fig 2 T to tip of III L, if not "pseudo translocation" From px bl

Fig 3 Df(3L)61C, 8 from px bl See tables 129, no 16, 46, no 11, 45, no 12

Fig 4 Df(2R)60F, 4-5, right of bs, ba, from px bl See table 129, no 14

Fig 5 I(?) near b-pr found in px bl, not main series

Fig 6 T(2L-3L) found in heterozygous rudimentary from px bl

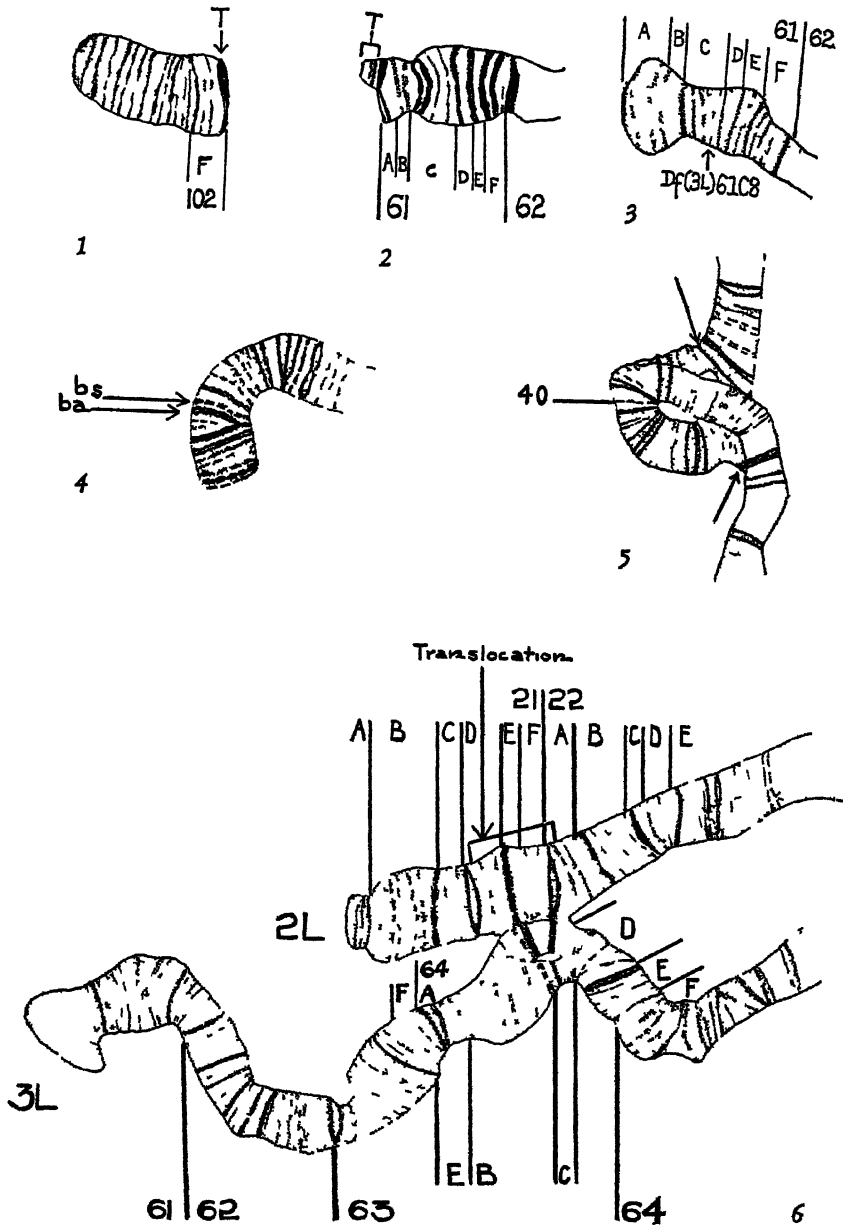
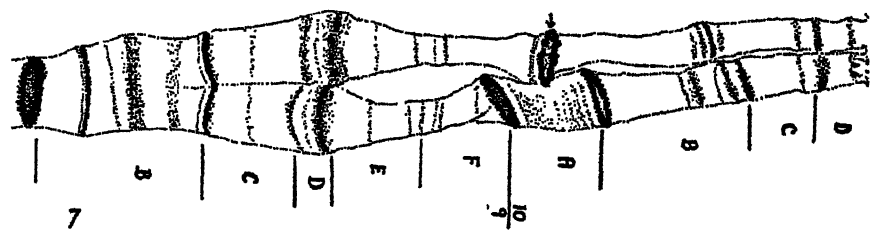
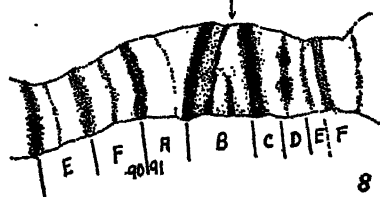
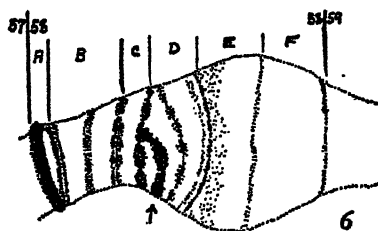
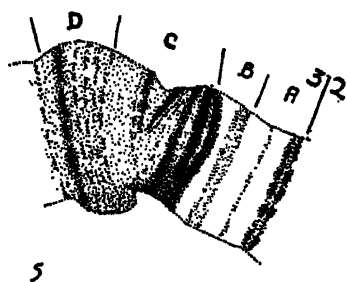
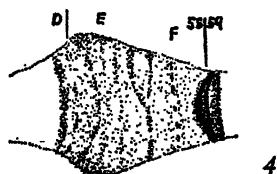
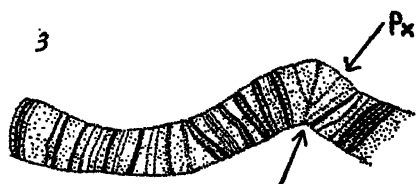
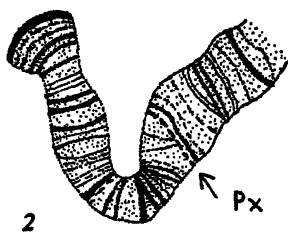
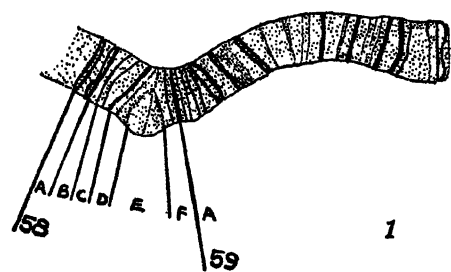


PLATE 28

Figs. 1-3, Kodani del.; others, Hannah del.

- Fig. 1. Tip second chromosome px bl homozygous.  
Figs. 2, 3, 4. Tip second chromosome px bl heterozygous.  
Fig. 5.  $svr^{pol}$  insertion in 1, 3C9.  
Fig. 6.  $svr^{pol}$  insertion 2B, 58D between 2 and 3.  
Fig. 7.  $svr^{pol} \cdot Df1$ , 9A1-2.  
Fig. 8.  $svr^{pol} \cdot h$  insertion 3R91B.





## PLATE 29

Hannah del

Fig 1 The arc region of *bran*<sup>2</sup> (combination *bran blist*). The 2 not synapsed chromosomes with *Df2R*, 58D6, 7

Figs 2, 3 Same region, synapsed both heterozygous

Fig. 4 *svr*<sup>po1</sup>/+. The region 1, 1E1-4 showing at some foci an apparent crossing of 1, 2 with 3, 4, explained in text as a 1 band inversion (of 3, 4). Four different foci drawn.

Fig 5 The same region in *poi sq*/+, two foci

Fig 6 The same region, *poi*/τ, 3 foci

Fig 7. The same region, 2 foci

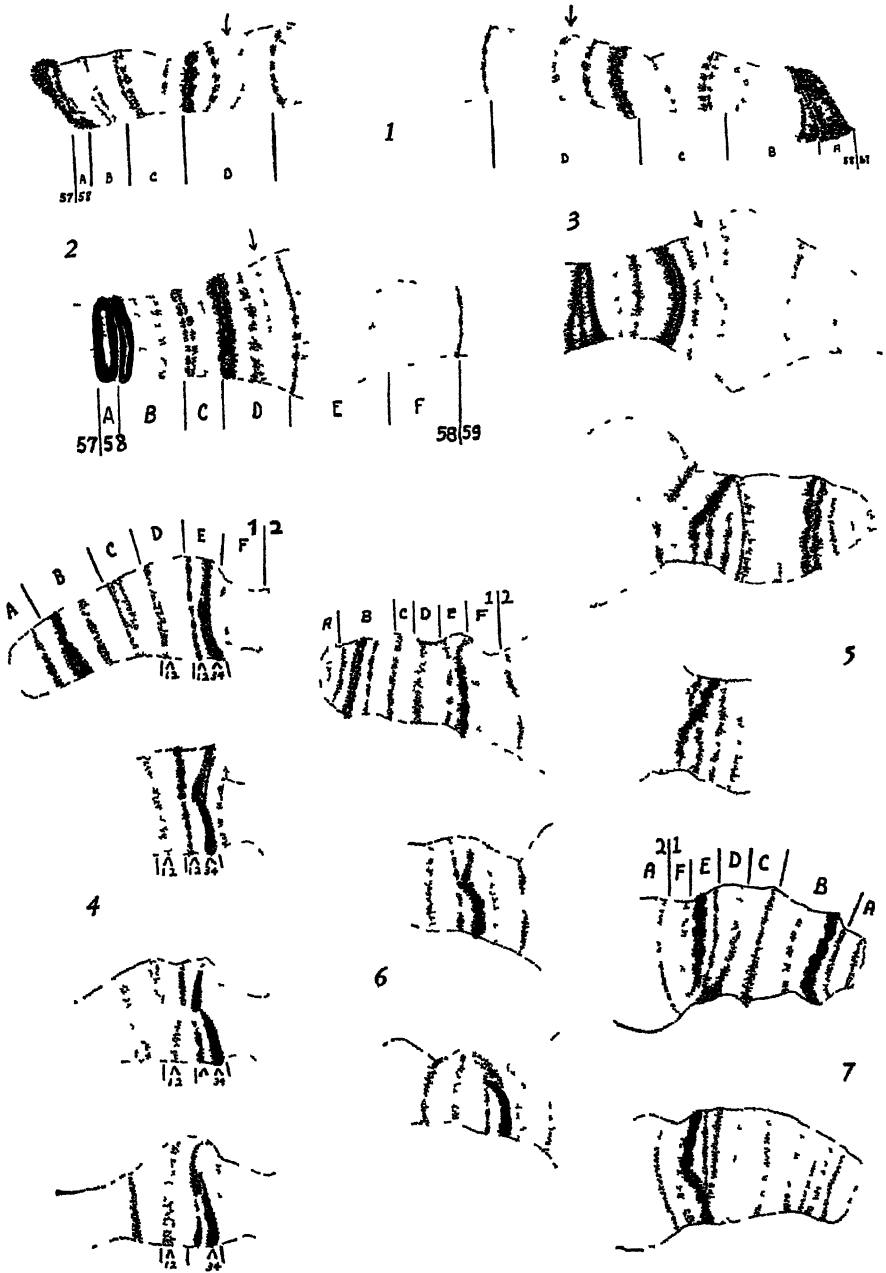


PLATE 30

Hannah del.

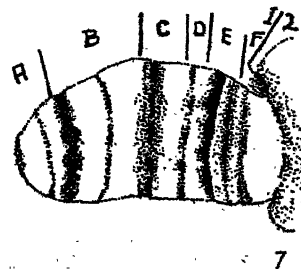
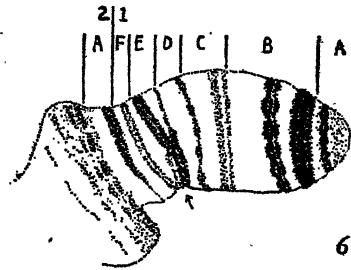
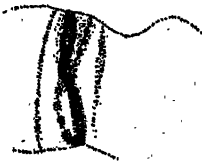
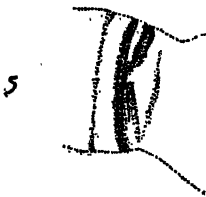
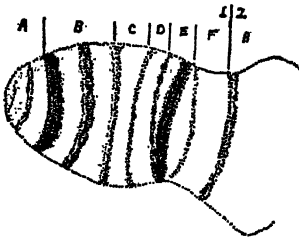
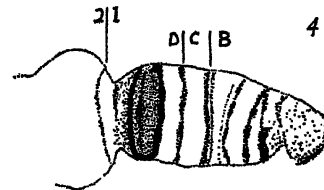
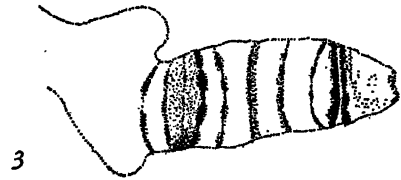
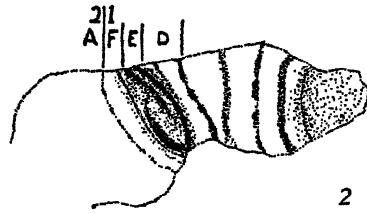
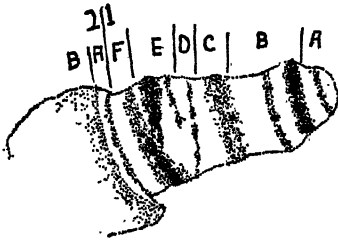
Figs. 1-4. Homozygous and heterozygous  $svr^{po1h}$  with unanalyzable features in 1, 1E1-4.

Fig. 1. Homozygous in 3 foci.

Figs. 2-4. Heterozygous.

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Fig. 7. The same region, homozygous, reversed order E3, 4-1, 2.





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